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Vitamin D: from pathophysiology to clinical impact, volume II

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Vitamin D: from pathophysiology to clinical impact, volume II

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Editorial: Vitamin D: from pathophysiology to clinical impact

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KEYWORDS

25(OH)D, extraskeletal districts, reference levels, threshold, vitamin D

Editorial on the Research Topic
Vitamin D: from pathophysiology to clinical impact

Besides the well-known positive effects on skeletal homeostasis and bone metabolism, growing evidence highlights the importance of vitamin D also in other many extra-skeletal districts; the articles published in this Research Topic confirm this observation in both adult and pediatric populations, spanning different conditions from inflammation and infectious diseases, obesity, and diabetes, to neurological disorders, gastrointestinal conditions, neurological disorders, cardiovascular health, and malignancies (see Table 1). This fact contributes to the increasing attention toward the measurement of serum 25(OH)D (main circulating form and recognized biomarker of vitamin D status) in laboratory medicine as well as the requirements of accuracy and speed of testing. However, despite the growth and refinement of analytical methods, the measurement of vitamin D still represents a challenge; immunoanalytical techniques still retain great variability in inter-laboratory comparisons, whereas mass spectrometry presents many different difficulties (e.g., costs, time, and complexity, matrix effects, derivatization step) (1). Interestingly, other vitamin D metabolites may have biological roles, and are expected to be assessed in the serum in the future (2).

In any case, at present the high prevalence of vitamin D deficiency is a worldwide major public sanitary issue in every stage of life, including children and adolescents, where an inadequate status can have implications on future health and wellbeing (3, 4). In this regard, as many as five contributions focusing on different children issues are included in this Research Topic (see Table 1). Moreover, available dosing recommendations for vitamin D supplementation may considerably vary in the literature depending on the clinical setting and specific cohort evaluated. Importantly, there is still no international general consensus on how to define an optimal vitamin D status, mainly defined based on the inverse relationship of parathyroid hormone and 25(OH)D. For it concerns bone health, the Endocrine Society defines as adequate serum 25(OH)D levels higher than 75 nmol/L (30 ng/mL) and values higher than 50 nmol/L (20 ng/mL) as deficient (5); instead, the Institute of Medicine (IOM, now National Academy of Medicine) definition suggests vitamin D sufficiency for values higher than 50 nmol/L (20 ng/mL), insufficiency between 30 and 50 nmol/L (12–20 ng/mL); deficiency for levels lower than 30 nmol/L (12 ng/mL) (6). In addition, differences may also be due to methodological

TABLE 1 Contributions of the Research Topic.

Title	Authors	Setting	Patients	Results
No causal relationship between serum vitamin D levels and alcoholic liver disease: a two-sample bidirectional Mendelian randomization study	Wu H, Wu L, Zhang Q, Li C, Li HY, Zhang BF.	Alcoholic liver disease (ALD)	Adults	No reciprocal causal link between serum VD and ALD susceptibility
Vitamin D in tuberous sclerosis complex-associated tumors	Tatsuro Nobutoki	Tuberous sclerosis complex-associated tumors	Children	Review discussing the possible role of 1,25VD in pediatric tuberous sclerosis complex-associated tumors, and the significance of vitamin D signaling as adjuvant or alternative drug target
Vitamin D status, vitamin D receptor, CYP2R1, and CYP24A1 profiles in children	Iriani A, Rachman A, Fatina MK, Gemilang RK, Trisnandi A, Muskananfola FV, Nugraha MFI.	Vitamin D status	Children	VD level is reduced by aging in children; VDR level is also found different based on VD status
Explorative case control study on the associations of serum vitamin D3, folic acid and vitamin B12 levels on Kawasaki disease and coronary artery lesions	Chen Y, Liu X, Li B, Li J, Meng L, Ye C, Han L, Li H, Deng LL, Su Z, Zhang X.	Kawasaki disease and coronary artery lesions	Children	Folic acid and VD3 were significantly reduced in children with KD, especially in those with coronary artery lesions
Changes in vitamin D status among adults from the COVID-19 pandemic to post-pandemic normality	Chen Y, Kong G.	COVID-19	Adults	COVID-19 pandemic significantly impacted VD status, leading to an increased prevalence of deficiency, especially among males
Nonlinear correlation and mediation effects between serum 25-hydroxyvitamin D levels and all-cause mortality in COPD patients	Jiang Q, Jiang Y, Ma Z, Huang J, Li Y.	COPD	Adults	High prevalence of low VD in COPD patients; non-linear association between serum 25(OH)D concentration and all-cause mortality (better survival above 63.40 nmol/L), with VD mediating role between dietary inflammation and mortality (mediation analysis)
The effects of vitamin D supplementation on serum lipid profiles in people with type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials	Lu Q, Liang Q, Xi Y.	Type 2 diabetes	Adults	The meta-analysis evidenced the benefit of VD supplementation in improving serum HDL and TG but not in LDL and TC levels
Associations of vitamin D status with all-cause and cause-specific mortality in long-term prescription opioid users	Dai S, Wu J, Wang P, Hu Z.	Opioid users	Adults	Non-linear association between serum 25(OH)D concentration and all-cause mortality
Implications of vitamin D levels or status for mortality in rheumatoid arthritis: analysis of 2001-2018 data from the National Health and Nutrition Examination Survey	Feng Y, Zhu P, Dandan Y, Wang X, Chen C, Zhang Z, Tian Y, Wang J, Liu S, Li J, Meng D, Wang K.	Rheumatoid arthritis (RA)	Adults	A significant negative correlation between 25(OH)D levels and overall mortality in individuals with RA, especially for mortality related to heart disease and cancer
Serum 25-hydroxyvitamin D concentrations and their impact on all-cause mortality in Parkinson's disease: insights from National Health and Nutrition Examination Survey 1999–2020 data	Yong Y, Dong H, Zhou Z, Zhu Y, Gu M, Li W.	Parkinson's disease	Adults	Non-linear association between serum 25(OH)D concentration and all-cause mortality (optimal survival rates at 75–100 nmol/L)
The complex relationship between vitamin D and kidney stones: balance, risks, and prevention strategies	Zhang F, Li W.	Kidney stones	Adults	The review discusses actual evidence on the relationship between vitamin D status and kidney stone risk, and the role of vitamin D supplementation for preventing and treating kidney stones.
Factors associated with vitamin D deficiency in health care workers exposed to SARS-CoV-2: a cross-sectional study	Villasis-Keever MA, Zurita-Cruz JN, Garduño-Espinosa J, López-Alarcón M, Barradas Vázquez AS, Miranda-Novales MG, Parra-Ortega I, López-Martinez B, García H, Klünder-Klünder M.	COVID-19	Adults	High prevalence of VD deficiency in Mexican health care workers exposed to SARS-CoV-2, related to both personal health factors and occupational low VD values is high among health care workers, and TD2 was a significant risk factor associated with these values. conditions (e.g. T2D)
The relationship between physical activity levels and serum vitamin D levels varies among children and adolescents in different age groups	Ouyang S, Li Q, Liu Z, Yin Y.	Physical activity (PA)	Children and adolescents	PA and VD varies according to sex and age, with sun exposure level and BMI affecting PA/VD relationship

(Continued)

TABLE 1 (Continued)

Title	Authors	Setting	Patients	Results
Comparative Analysis of COVID-19 Responses in Japan and Africa: Diet, Phytochemicals, Vitamin D, and Gut Microbiota in Reducing Mortality -A Systematic Review and Meta-Analysis	Santa K, Tamaki R, Watanabe K, Nagaoka I.	COVID-19	Adults	Blood vitamin D levels are associated with COVID-19 mortality
Vitamin D Deficiency in Non-scarring and Scarring Alopecias: A Systematic Review and Meta-Analysis	Yongpisarn T, Tejapira K, Kunlawat Thadanipon K, Suchonwanit P.	Alopecias	Adults	Patients with non-scarring alopecia have insufficient serum VD level and increased incidence of vitamin D deficiency
Cardiometabolic factors and vitamin D deficiency in pediatric patients with chronic kidney disease	Fayoumi T, Gari A, Alarawi M, Almutairi S, Shalabi BH, Safdar O, Al Kadi H.	Chronic kidney disease (CKD)	Children	Children with CKD and low VD ($\leq$41.9 nmol/L) presented a more adverse cardiometabolic risk profile
Relationship between Serum Vitamin D Levels and the Atherogenic Index of Plasma (AIP): A Study Based on NHANES Database 2011–2018	Hu T, Zhang Y, Chen Z, Su J.	Risk of atherosclerosis	Adults	A significant negative correlation and saturation effect was found between serum vitamin D and the atherogenic index of plasma (AIP) in a large general adult population ($n = 9637$) from the NHANES

VD, vitamin D; VDR, vitamin D receptor; T2D, type 2 diabetes; BMI, body mass index; COPD, chronic obstructive pulmonary disease; KD, Kawasaki disease.

issues (e.g., seasonal sampling, variability in the vitamin D assays), characteristics of the studied populations, (e.g., age, sex, and calcium intake), and life-style habits (physical activity, outdoor activities, and sun exposure) (1). Moreover, the majority of evidence derived from studies conducted in adults, and even the threshold used to define vitamin D deficiency or insufficiency may not be appropriate when applied to children and/or adolescents. Indeed, currently, there are no specific guidelines and no clear consensus on targets for optimal vitamin D status and supplementation in most extra-skeletal conditions (1). Thus, for non-classical actions, more research is needed concerning the optimal 25(OH)D levels to maintain and clarify the suitability of 25(OH)D reference levels (in turn useful to decide the recommended vitamin D intake to maintain most people above this threshold, providing adequacy) and the role of vitamin D as key indicator of health in different clinical settings. We hope that manuscripts included in this Research Topic, which highlight the role and levels of vitamin D in different pathophysiological conditions, may contribute to the actual discussion in this field and may be appreciated by readers of Frontiers in Nutrition, facilitating further studies stimulated by the data reported and by the many challenges still to be solved in this important research area.

Author contributions

CV: Conceptualization, Writing – original draft.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Vitamin D in tuberous sclerosis complex-associated tumors

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Mammalian target of rapamycin inhibitors (mTORi) have been used to treat pediatric tuberous sclerosis complex (TSC)-associated tumors, particularly in cases with contraindications to surgery or difficulties in complete tumor resection. However, some patients experience side effects and tumor regression after discontinuation of the treatment. Therefore, there is an urgent need to develop drugs that can be used in combination with mTORi to increase their efficacy and minimize their side effects. 1,25-Dihydroxyvitamin D_3 (1,25-D), which has anticancer properties, may be a promising candidate for adjuvant or alternative therapy because TSC and cancer cells share common mechanisms, including angiogenesis, cell growth, and proliferation. Vitamin D receptor-mediated signaling can be epigenetically modified and plays an important role in susceptibility to 1,25-D. Therefore, vitamin D signaling may be a promising drug target, and *in vitro* studies are required to evaluate the efficacy of 1,25-D in TSC-associated tumors, brain development, and core symptoms of psychiatric disorders.

KEYWORDS

tuberous sclerosis complex, tumor, 1,25-Dihydroxyvitamin D_3, vitamin D receptor, mTOR

1 Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by inactivating mutations in either the *TSC1* or *TSC2* gene, which affects multiple organ systems and results in various clinical features (1). *TSC1* encodes *hamartin* and *TSC2* encodes *tuberin*. Since TSC1 and TSC2 are tumor suppressor genes, abnormalities in *hamartin* or tuberin lead to mammalian target of rapamycin (mTOR) overactivation and multisystemic cellular proliferation, migration, and differentiation abnormalities (1). The role of mTOR inhibitors (mTORi) in cancer (2) and their safety in TSC (3) have been established. However, for children with TSC-associated tumors, it is critical to have an alternative therapeutic option when mTORi are ineffective or cannot be used.

1,25-Dihydroxyvitamin D_3 (1,25-D) activates DNA damage-inducible transcript 4 (DDIT4) (4), which activates the TSC1-TSC2 complex and ultimately represses mTOR (5). Daily vitamin D supplementation was shown to reduce overall cancer mortality (6). An association has been suggested between cancer and low levels of circulating 25-hydroxyvitamin D_3 (25-D) in ovarian (7), prostate (8), and colorectal cancers (9).

Abbreviations

1,25-D, 1,25-dihydroxyvitamin D_3; 25-D, 25-hydroxyvitamin D_3; Akt, RAC-alpha serine/threonine-protein kinase; ASD, autism spectrum disorder; CaM, calcium/calmodulin; DDIT4, DNA damage-inducible transcript 4; ERK, extracellular signal-regulated kinase; GB, glioblastoma; HDAC, histone deacetylase; HIF-1α, hypoxia-inducible factor-1α; IκB, kinase β; MAPK, Mitogen-activated protein kinase; miRNA, microRNA; mTOR, mammalian target of rapamycin; mTORi, mTOR inhibitors; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue; S6K, S6Kinase; SEGA, subependymal giant cell astrocytoma; TrkB, tyrosine receptor kinase B; TSC, tuberous sclerosis complex; VDR, vitamin D receptor; VEGF, vascular endothelial growth factor; VM, vasculogenic mimicry; Wnt, wingless/int-1.

However, whether vitamin D can prevent cancer remains controversial. Therefore, it is important to investigate whether 1,25-D not only serves as an adjuvant therapy by inhibiting or reducing TSC-associated tumor cell proliferation but also improves mTORi tolerability.

In addition to its effects on mTOR suppression, 1,25-D negatively regulates energy metabolism in cancer cells, including glucose and lipid metabolism, protection from oxidative stress, and cancer progression (10). Higher serum 25-D levels are associated with a reduced risk of glioma in elderly men (11). Recent results from an *in vitro* study have led to a discussion on the potential clinical use of vitamin D for treating glioblastoma (12). Thus, 1,25-D may be a weak suppressor of TSC-associated tumor growth, including in cases of drug resistance and rapidly growing subependymal giant cell astrocytoma (SEGA). This article provides perspectives on the potential adjuvant therapy using 1,25-D in patients with TSC, along with a review and presentation of hypotheses associated with the underlying physiological mechanisms.

2 Need for an alternative or supportive drug to mTORi in pediatric TSC

In pediatric patients with TSC, SEGA (in ages ≥1 year) and refractory partial-onset seizures (as an adjunctive treatment, in ages ≥2 years) can be treated with mTORi (13). However, side effects are more common in children than in adults (13). The overall incidence of adverse events in children aged <9 years was 70.5% (24 of 34 patients), of which 33.3% (8 of 24 patients) had grade 3 side effects (13). Moreover, the mechanism of mTOR resistance in each tumor type has not been elucidated yet. Therefore, in addition to investigating the resistance mechanism, there is an urgent need to identify safe and effective drugs that can support mTORi treatment, including drug repurposing and combination therapy.

3 1,25-D inhibits vascular endothelial growth factor (VEGF) production and early angiogenesis in TSC-associated tumors

1,25 D causes transcriptional changes by binding to the intracellular vitamin D receptor (VDR). This binding forms a complex that interacts with specific DNA sequences called vitamin D-response elements (VDREs) located within the promoter regions of target genes (14).

1,25-D activates the production of the DDIT4 protein, which is induced by hypoxia and DNA damage via intracellular VDR (5). The DDIT4 inhibits mTOR complex 1 by promoting TSC1-TSC2 complex formation (15) (Figure 1A). Moreover, the DDIT4-TSC1/TSC2-mTOR feedback loop downregulates the production of hypoxia-inducible factor-1α (HIF-1α) and VEGF (Vascular Endothelial Growth Factor) (15). siRNA knockdown of DDIT4 eliminates the antiproliferative effect of 1,25-D (16). Thus, 1,25-D restricts HIF-1-dependent VEGF production in various human cancer cells under hypoxic conditions (17) and induces apoptosis in existing sprouted and elongated endothelial cells (18) Notably, TSC-associated tumors are angiogenic neoplasms (19) expressing high levels of VEGF (20). Through this mechanism, 1,25-D may suppress tumor cell proliferation by inhibiting the excessive activation of the HIF/VEGF pathway in the vasculature of TSC-associated tumors.

Moreover, activation of VEGF, HIF, and endothelial cell-dependent mechanisms contribute to vasculogenic mimicry (VM) in cancer (21). This involves the formation of microvascular channels composed of tumor cells that contribute to the resistance to anti-angiogenic therapy (22). Hypoxic conditions and the aberrant activation of the mTOR/HIF/VEGF pathway can occur in cancer and TSC-associated tumors. Indeed, astrocytomas have a VM-like structure (23), and VEGF- and HIF-dependent factors play an important role in their pathogenesis (20). This mechanism may contribute to therapy resistance in SEGA and to tumor regrowth after discontinuation. Therefore, it is reasonable to target the HIF/VEGF pathway with 1,25-D since a VM-like structure may be formed in TSC-associated tumors, including SEGA.

TSC1 is suppressed by IκB kinase β (IKKβ) phosphorylation, leading to the activation of the mTOR pathway and increased VEGF production and angiogenesis (24). 1,25-D induces direct VDR-IKKβ protein interaction, disrupting the formation of the IKK complex, which consists of IKKα, β, and γ subunits, and abolishes IKKβ phosphorylation (25) (Figure 1B). Through this mechanism, 1,25-D suppresses VEGF-mediated angiogenesis.

4 Impaired interaction between TSC2 and VDR may contribute to TSC severity

In tumor-derived endothelial cells, the transcription of one of the VDR target genes, 24-hydroxylase (CYP24A1) is upregulated and this promotes the metabolism of 25-D and 1,25-D. The activation of CYP24A1 promotes 25-D metabolism, thereby potentially reducing the cellular availability of 1,25-D. Moreover, cell cycle arrest, growth inhibition, and apoptosis are induced by epigenetic silencing of CYP24A1 (26). Importantly, plasma membrane-bound TSC2 binds to calcium/calmodulin (CaM), and this complex is translocated to the nucleus, partially attenuating CYP24A1 transcription under normal conditions (27) (Figure 1C1). Mutations in TSC2 lead to more severe clinical features compared to those in TSC1 This, in turn, could contribute to the severity of TSC and the formation of TSC-associated tumors. This, in turn, could contribute to the severity of TSC and the formation of TSC-associated tumors. Therefore, along with inhibiting angiogenesis and regressing existing immature capillaries, 1,25-D treatment produces effects in tumor cells similar to those of epigenetic silencing of CYP24A1.

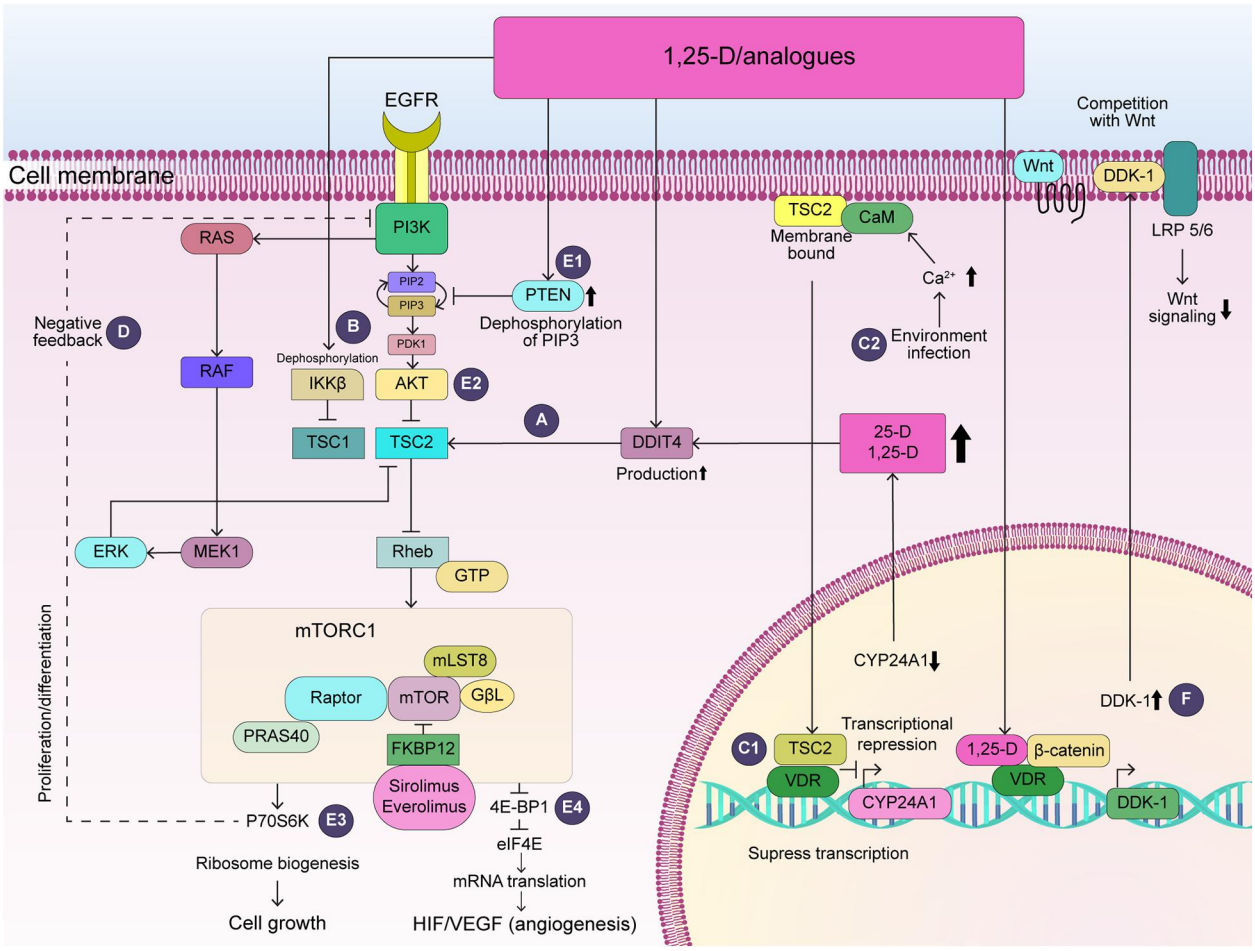

FIGURE 1
Potential action of vitamin D in TSC-associated tumors. (**A**) DDIT4 activates TSC2, which inhibits Rheb and ultimately suppresses mTOR activity. (**B**) 1,25-D dephosphorylates and inactivates IKKβ, which inhibits TSC1. (C1) TSC2-CaM complex translocates into the nucleus and represses CYP24A1 transcription. (C2) Increased intracellular calcium, which activates CaM by environmental factors including viral infection, could increase attenuation of transcription of VDR-sensitive genes. (**D**) S6K activation suppresses the PI3K/AKT and ERK pathway via negative feedback. (**E**) 1,25-D activates the production of PTEN (E1), which inhibits PI3K, leading to the dephosphorylation of Akt (E2), ultimately reducing p70S6K activity (E3) and 4E-BP1 (E4). Thus, 1,25-D may contribute to the suppression of cell growth and angiogenesis in TSC-associated tumors. (**F**) 1,25-D increases VDR/β-catenin binding, which increases transcription of the VDR-sensitive gene, DDK-1, competing with Wnt and decreasing Wnt signaling. AKT, RAC-alpha serine/threonine-protein kinase; CaM, calcium-calmodulin; CYP24A1, 24-hydroxylase; 1,25-D, 1,25-dihydroxyvitamin D_3; DDK-1, Dickkopf-1; DDIT4, DNA damage inducible transcript 4; Deptor, DEP-domain-containing mTOR-interacting protein; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; eIF4E, eukaryotic initiation factor 4E; EGFR, epidermal growth factor receptor; ERK1-2, extracellular signal-related kinase; FKBP12, FK506-binding protein with a molecular weight of 12 kDa; GβL, G protein β subunit-like protein; GTP, guanosine triphosphate; HIF-1α, hypoxia inducible factor-1-alpha, IKKβ, IκB kinase β; Lamptor, late endosomal/lysosomal adaptor, mitogen-activated protein kinase, and mTOR activator; LRP5/6, low-density lipoprotein receptor-related proteins 5 and 6; MEK1-2, mitogen-activated protein kinase 1-2; mLST8, mammalian lethal with SEC13 protein 8; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; P70S6K, 70-kDa ribosome protein S6Kinase; PDK1, 3-phosphoinositide-dependent protein kinase-1; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PI3K, phosphoinositide 3-kinase; PRAS40, proline-rich Akt substrate of 40 kDa; PTEN, phosphatase and tensin homologue; RAF, rapidly accelerated fibrosarcoma viral oncogene homologue; RTK, rector of tyrosine kinase; Raptor, regulatory associated protein of mTOR complex 1; RAS, rat sarcoma viral oncogene homologue; Rheb, Ras homologue enriched in the brain; TSC, tuberous sclerosis complex; VDR, vitamin D receptor; VEGF, vascular endothelial growth factor.

5 Second hit by epigenetic alterations in the vitamin D metabolism and VDR signaling pathway may be associated with clinical features in TSC

The correction of histone hyperacetylation in hippocampal neurons using histone deacetylase (HDAC) inhibitors improves abnormal synaptic plasticity and epilepsy in TSC (28). While second-hit mutagenesis may play an important role in the phenotypic diversity of renal lesions in TSC (29), the VDR and VDR-responsive genes can be epigenetically modified (30), similar to histone hyperacetylation in neurons. Therefore, postnatal epigenetic modifications of VDR-mediated signaling and vitamin D metabolism may serve as a second hit and contribute to the clinical variability and severity of TSC, including the development of tumors, abnormal synaptic plasticity, and epilepsy. As discussed

above, CaM plays an important role in the TSC2-VDR interaction. Therefore, increased intracellular calcium and CaM activation caused by viral infection could increase the attenuation of VDR-sensitive gene transcription, including that of CYP24A1, by enhancing the TSC2-CaM interaction or inducing epigenetic modifications that affect the transcriptional activity of VDR-related genes (Figure 1C2).

Epigenetic silencing of VDR, which is also mediated by HDAC3, is crucial in 1,25-D resistance (30), and the treatment of VDR promoter hypermethylation with 5-aza-2′-deoxycytidine restored VDR mRNA expression (31). In addition, hypermethylation of CYP27B1, which activates 25-D to 1,25-D, can reduce the tissue levels of 1,25-D (30). High blood 25-D and 1,25-D levels may be required in this condition.

6 SEGA responds to 1,25-D under increased VDR expression and sensitivity

Although VDR expression is increased in human glioblastoma (GB) cells (32), high VDR expression in GB is associated with 1,25-D treatment success (33). In an *in vitro* study, calcitriol and vitamin D analogue blocked the stem-like properties of glioma cells (34), induced DNA fragmentation (35), and inhibited the migration of GB cells (33). Furthermore, 1,25-D restores responsiveness to itself by upregulating VDR expression (36). Based on these observations, VDR expression in SEGA potentially correlates with 1,25-D sensitivity or aggressiveness. It's also speculated that SEGA, exhibiting resistance or recurrence to mTOR inhibitors, may respond to 1,25-D similar to GB.

Vitamin D_3 analogue can suppress the activation of the phosphatidylinositol 3-kinase (PI3K)/RAC-alpha serine/threonine-protein kinase (AKT) pathway and extracellular signal-regulated kinase (ERK)/Mitogen-activated protein kinase (MAPK) pathway due to mTORi therapy.

S6Kinase (S6K) activation via mTORC1 suppresses the PI3K/AKT and ERK pathways via negative feedback (Figure 1D). Therefore, S6K inhibition by mTORi contributes to the activation of both pathways, thereby contributing to the pathology of mTORi-resistance. Therefore, combination therapy with MAPK inhibitors may be useful (37). Notably, vitamin D and its analogs restrict gliomas by inducing cell cycle arrest via multiple mechanisms, including the PI3K/AKT pathway (12). Importantly, while therapy with mTORi and PI3Kα inhibitor is necessary for GB treatment (38), activation of the PI3K/AKT (39) and MAPK/ERK pathways (40) also plays an important role in SEGA development. Moreover, in TSC, the Raf-1/MAPK/ERK cascade, in addition to mTOR, leads to 4E-binding protein 1 phosphorylation, increased cyclin D protein levels, and increased protein synthesis (41). The active vitamin D analog, maxacalcitol, decreased hyperphosphorylation of MAPK-38p and ERK1/2 in the brain tissue of a mouse model of Alzheimer's disease (42). Therefore, the vitamin D_3 analog may suppress the growth of renal cancer and SEGA in the same way. Particularly in patients with mutations in the tumor suppressor gene phosphatase and tensin homologue (PTEN), PI3K/AKT activation leads to tuberin phosphorylation and decreased activity of tuberin-hamartin complex, resulting in the activation of mTOR/70kDa-S6K1 signaling (43). A novel vitamin D_3 analog, Gemini-23-yne-26,27-hexafluoro-D_3, not only increased the expression of PTEN and caused the dephosphorylation of Akt, Ark target proteins, and mTOR, but also decreased the phosphorylation of its downstream effectors, S6Ks, and eukaryotic translation initiation factor 4E-binding protein 1, thereby suppressing protein synthesis and tumor proliferation (44) (Figures 1E1–E4). Similarly, tumors associated with Tuberous Sclerosis Complex (TSC), such as SEGA, might see improved treatment outcomes with novel vitamin D derivatives. These derivatives activate PTEN, suppress the PI3K/AKT/mTOR pathway, and enhance the effectiveness of mTOR inhibitors, potentially leading to safer and more effective therapies compared to using mTOR inhibitors alone.

7 Suppressing wingless/int-1 (Wnt)/β-catenin signaling

Abnormal Wnt/β-catenin signaling plays an important role in tumorigenesis (45). The TSC1-TSC2 complex negatively regulates cell proliferation through β-catenin signaling (46), which plays an important role in the pathogenesis of angiomyolipomas and lymphangioleiomyomatosis (47). Importantly, 1,25-D increases VDR/β-catenin binding, which, in turn, increases the transcription of one of the VDR target genes, the Wnt inhibitor Dickkopf-1, to a greater degree than that of the T-cell factor (48) (Figure 1F). As a result, 1,25-D decreases the transcription of β-catenin/T-cell factor-target genes that regulate cell proliferation, cell cycle regulation, and cellular metabolism (48). Therefore, 1,25-D may inhibit the growth of TSC-associated tumors.

8 Identification of 1,25-D-induced microRNAs (miRNAs) that suppress the growth of TSC-associated tumors and re-sensitize mTORi-resistant tumors

miRNAs are short noncoding RNAs with a wide range of gene regulatory activities at the post-transcriptional level (49). 1,25-D re-sensitizes everolimus-resistant hepatocellular carcinoma by upregulating miRNA-375, which regulates the oncogenes responsible for drug resistance (50). In addition, miRNA-22 mediates the suppression of several genes by 1,25-D, contributing to its antiproliferative and antimigratory effects in colon cancer cells (49). Therefore, it's worth exploring if 1,25-D also triggers miRNA-mediated antitumor effects in TSC and enhances the effectiveness or sensitivity to mTORi.

9 Initiating 1,25-D therapy in infancy improves brain development in TSC

Vitamin D is essential for brain development (51), and loss of TCS2 function in brain endothelial cells, neurons, oligodendrocytes,

astrocytes, and microglia may increase the degradation of vitamin D and decrease its bioavailability by the same mechanism discussed above. Therefore, central nervous system vitamin D deficiency in TSC might be one of the drivers of impaired neural and synaptic maturation, as well as of tumorigenesis. Notably, blood levels of 25-D higher than 40 ng/ml can improve the autism spectrum disorder (ASD) rating (52). Thus, the same therapeutic effects can be expected in TSC. If the sensitivity of neural and brain endothelial cells to vitamin D is increased, early vitamin D treatment from infancy can improve brain development and pediatric TSC-associated neuropsychiatric disorders, including the core symptoms of ASD and refractory epilepsy.mTOR-dependent synaptic hyperconnectivity is implicated in ASD pathogenesis in *Tsc2*$^{+/-}$ mice (53). mTORi are effective in rescuing synaptic hyperconnectivity and controlling autistic behavior (53) and epilepsy in patients with TSC (54).

Neurological problems can be associated with decreased 1,25-D bioavailability in neurons and neuronal unresponsiveness to 1,25-D in the developing brain, owing to the epigenetic silencing of VDR-mediated signaling. In either case, the combination of mTORi and 1,25-D may synergistically improve brain development.

In addition, 1,25-D inhibits IKKβ phosphorylation and suppresses nuclear factor-κB (25), which plays an important role in the switch from oxidative stress to inflammation that contributes to epileptogenesis (55). Thus, 1,25-D may be promising for the reversal of TSC-associated brain pathological conditions and may play a role in suppressing the development of SEGA and reducing the clinical severity of comorbid neurological disorders.

10 Potential considerations in vitamin D treatment: therapy resistance and tumor growth

Since mTOR inhibition provides survival advantage for tumor cells (56), it is important to consider the risk of 1,25-D increasing tumor growth by inhibiting mTOR through DDIT4 activation. In silico analysis has shown that high DDIT4 expression, but not PI3K/mTOR activation, is associated with poor prognosis in some cancers, suggesting that DDIT4 inhibitors may be effective in these cases (56).

Tumor hypoxia, a condition in which solid tumors have hypoxic regions with an insufficient oxygen supply, contributes to resistance to chemotherapy (57). This may also occur in TSC-associated tumors. Under hypoxia, HIF-1α production is activated, which transcriptionally upregulates DDIT4 through a negative feedback loop of suppression against mTOR inhibition (15). Thus, when DDIT4 is overexpressed in TSC-associated tumors, mTOR/HIF-1α may persistently be overactivated by this mechanism, leading to a resistance to mTORi and 1,25-D. Under these conditions, DDIT4 inhibitors, but not 1,25-D, can minimize the dose of mTORi and avoid the need to discontinue treatment owing to side effects. Therefore, *in vitro* studies are important to determine the effectiveness of 1,25-D or its potential to exacerbate tumor progression, particularly in cases of high DDIT4 levels within the tumor tissue.

Moreover, while cholecalciferol exerts beneficial antidepressant effects through the activation of brain-derived growth factor/tyrosine receptor kinase B (TrkB) signaling in the prefrontal cortex (58), TrkB signaling plays an important role in TSC-associated neuropsychiatric disorders and epileptogenesis (59). While activation of the brain-derived neurotrophic factor/TrkB pathway by early 1,25-D therapy may improve brain development in children with TSC, epidemiological studies are required to investigate whether long-term 1,25-D treatment may improve neuropsychiatric disorders and epilepsy.

11 Epidemiological studies using large medical datasets may be useful for testing hypothesis

Considering the above discussion, it cannot be excluded that the clinical phenotypic variability in TSC, including tumor development, progression, and neurological problems, is partly due to the second hit in epigenetic modification of VDR and vit D-metabolizing enzyme genes by the environment, as well as dietary and supplemental vitamin D intake, use of multiple anticonvulsants, and reduced sun exposure. It is necessary to investigate whether 1,25-D treatment can reduce the risk or worsen the severity of TSC-associated tumors, refractory epilepsy, and neuropsychiatric conditions, including the core symptoms of ASD.

Analyzing medical big data or conducting retrospective studies on tumors, cancer incidence, prognosis, cognitive performance, and epilepsy severity in patients who received 1,25-D therapy compared to those who didn't, including assessing blood 25-D levels and the duration of 1,25-D therapy, may help determine if early and long-term treatment can benefit patients with TSC and TSC-associated tumors. Additionally, comparing the efficacy and side effects of mTORi alone vs. in combination with 1,25-D could provide valuable insights. However, conducting prospective studies raises ethical concerns due to the essential role of vitamin D in bone growth and immunity. Therefore, the analysis of medical big data could contribute to the discussion of the advantages and disadvantages of early 1,25-D therapy in patients with TSC.

12 Discussion

Since vitamin D has beneficial effects on several signaling pathways involved in the mechanism of TSC-associated tumors, 1,25-D and its analogs may be the first treatment choice. In children with TSC who have undertaken polytherapy of antiepileptic drugs, if vitamin D supplementation can not only prevent or slow tumor development but also improve brain development and reduce the core symptoms of TSC-associated neuropsychiatric disorders, it should be started immediately after diagnosis, preferably in infancy, in and added to anticonvulsant therapy. In addition, the possible increased

metabolism of 25-D in TSC-associated tumors and the downregulation of the transcriptional activity of VDR-sensitive genes at a relatively high dose (blood levels of 25-D > 40 ng/ml) seems reasonable. As 1,25-D is relatively inexpensive and safe, children who are intolerant to mTORi, those in long-term care facilities, those receiving home care, or those unable to receive expensive medical care may benefit from 1,25-D therapy. Thus, 1,25-D may be a promising drug candidate for enhancing the effects of mTORi and improving tolerability, although it is also important to study whether it has any side effects. Particularly, the study of VDR signaling, including the epigenome, in TSC may have implications for drug discovery. Therefore, it is necessary to study vitamin D signaling and search for novel vitamin D analogs that are more effective and have fewer side effects, such as hypercalcemia in TSC-associated tumors.

In vitro studies are required to evaluate the efficacy of 1,25-D in TSC associated tumors, brain development, and core symptoms of psychiatric disorders.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

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Conflict of interest

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Nonlinear correlation and mediation effects between serum 25-hydroxyvitamin D levels and all-cause mortality in COPD patients

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Background: Numerous studies have shown that low levels of vitamin D are linked to a higher risk of inflammatory diseases and their progression. However, how vitamin D levels affect mortality in chronic obstructive pulmonary disease (COPD) patients is still unclear. Thus, this study aimed to explore the relationship between serum 25-hydroxyvitamin D [25(OH)D] levels and the risk of death from all causes in U.S. adults with COPD.

Methods: This study analyzed 1,876 adults with COPD from the National Health and Nutrition Examination Survey (2005–2018). Mortality data up to December 31, 2019, were obtained from the National Death Index (NDI) records. Participants were categorized into three groups according to their 25(OH)D levels: Q1 (<50.0 nmol/L) for deficiency; Q2 (50.0–74.9 nmol/L) for insufficiency; and Q3 (≥75.0 nmol/L) for adequacy. A weighted Cox regression model assessed the link between 25(OH)D levels and mortality. Kaplan–Meier survival curves, subgroup, and sensitivity analyses were conducted. Additionally, the relationship between 25(OH)D and the hazard ratio (HR) was detailed through restricted cubic spline analysis. Mediation analysis revealed how 25(OH)D mediates the relationship between Dietary Inflammatory Index and mortality.

Results: There were 395 all-cause deaths during the follow-up, resulting in a mortality rate of 21.06%. After adjusting for potential confounders, higher 25(OH)D levels significantly correlated with a lower risk of all-cause mortality in COPD patients (HR = 0.52, 95% CI: 0.37–0.72, $p < 0.001$). Restricted cubic spline analysis indicated a non-linear relationship between 25(OH)D levels and all-cause mortality (p for nonlinear = 0.023), with levels below 63.4 nmol/L posing an independent risk for all-cause mortality in COPD patients (HR = 0.98, 95% CI: 0.97–0.99, $p = 0.005$). Sensitivity and subgroup analyses confirmed our results' robustness, with mediation analysis showing 25(OH)D's 22% mediating effect on diet-induced inflammation and all-cause mortality in COPD patients.

Conclusion: 25(OH)D independently lowers the risk of all-cause mortality in COPD patients, with a non-linear L-shaped correlation, and mediates the effect of Dietary Inflammatory Index on mortality, suggesting new therapeutic possibilities.

KEYWORDS

COPD, mortality, vitamin D, cohort study, NHANES

Background

Chronic obstructive pulmonary disease (COPD) has the highest mortality rate among chronic respiratory diseases (1). According to the World Health Organization, COPD is projected to become the third leading cause of death globally and the fourth leading cause of death in the United States in the next decade. The disease burden caused by COPD is expected to slowly and steadily increase (2, 3). Characterized by persistent airflow limitation and chronic inflammation of the airways, COPD ranks among the leading causes of mortality and morbidity, resulting in a substantial socioeconomic burden (4). To date, the pathological mechanisms of COPD are attributed to excessive inflammation, dysfunctional oxidative stress, and imbalance of protease-antiprotease systems (5–7).

As a type of steroid hormone, vitamin D can have unique biological effects on many target organs. It is most well-known for its role in bone calcium metabolism and maintaining the homeostasis of bone and calcium (8). Additionally, many studies also have revealed the unique effects of 25-hydroxyvitamin D on various cellular processes, such as cell proliferation, differentiation, wound healing, repair, and involvement in the host immune and inflammatory regulatory systems (9). The deficiency of 25-hydroxyvitamin D has been confirmed to be associated with the progression of multiple COPD pathogenesis processes, including inflammation regulation, excessive oxidative stress, increased protease expression, impaired host defense, and pulmonary airway remodeling (10). Evidence from clinical trials and meta-analyses indicates that 25-hydroxyvitamin D supplementation plays a role in reducing COPD exacerbations and improving disease prognosis (11–14).

Previous studies have highlighted vitamin D's potential to reduce mortality rates across diseases and its role in delaying COPD progression (15, 16). However, there is limited current scientific research on the association between vitamin D and mortality rates in COPD patients (17, 18). Specifically, no comprehensive study has explored the direct effect of vitamin D on mortality risk in COPD patients among the non-institutionalized population in the United States. This gap is critical for understanding vitamin D's role in COPD management because the non-institutionalized population represents a wider range of COPD patients, making the research findings more applicable and practical. This study will analyze NHANES data from 2005 to 2018 to investigate the relationship between serum 25-hydroxyvitamin D levels and all-cause mortality in COPD patients, as well as explore the potential impact of varying levels of serum 25-hydroxyvitamin D on mortality in COPD patients. The NHANES database, which contains nationally representative samples, offers a unique opportunity to address this research gap. The database covers a diverse population and ensures data accuracy and quality through standardized data collection processes. This solidifies the foundation for conducting reliable and effective statistical analyses. This study aims to fill gaps in the existing literature and enhance understanding of vitamin D's potential role in reducing mortality risk among COPD patients.

Methods

Study design and data source

This study analyses data from the US National Health and Nutrition Examination Survey (NHANES) database, focusing on the years 2005–2018. The primary objective is to explore associations between serum 25-hydroxyvitamin D levels and long-term mortality rates in community-dwelling adults with COPD. Additionally, the study examines the potential mediating role of serum vitamin D in the relationship between the dietary inflammation index and mortality.

The NHANES database comprises data collected by the National Center for Health Statistics (NCHS), part of the CDC in the United States. The survey is designed to assess the health and nutritional status of individuals from various age groups nationwide. A sophisticated, multistage design in survey procedures ensures the data's representativeness of the US population, excluding those in institutional settings. Researchers are granted access to use the data for research, made available by the NCHS.

NHANES survey participants first undergo a household interview and are then invited for a comprehensive examination at a mobile examination center (MEC). The examination encompasses physical measurements, specialized tests, and lab assessments. Consequently, participant evaluations from the NHANES database are deemed reliable and comprehensive, similar to population-level assessments (19). More information on the NHANES survey is available at its official website: https://www.cdc.gov/nchs/nhanes/index.htm. Note that all participants provided written informed consent for the NHANES survey.

Study population selection

The study included community-dwelling adults aged 40–79, diagnosed with COPD by a physician between 2005 and 2018 in the NHANES dataset. COPD identification from the NHANES questionnaire was based on affirmative responses to questions about a doctor's diagnosis of COPD, chronic bronchitis, or emphysema. This method for identifying COPD patients has been used effectively in many previous studies using NHANES data (20, 21). Participants who answered "yes" to any of these questions were considered COPD patients. From the initial 70,190 participants, 1,876 were included in the study after excluding those without vitamin D levels, survival data, or covariates, and those who could not be diagnosed with COPD. The sample is representative of 11,221,247 individuals in the United States, with the screening process detailed in Figure 1.

Mortality status

Since late 2019, NHANES participants have been linked to the National Death Index (NDI) database, which contains nine cause-specific death categories. This link facilitates the identification of

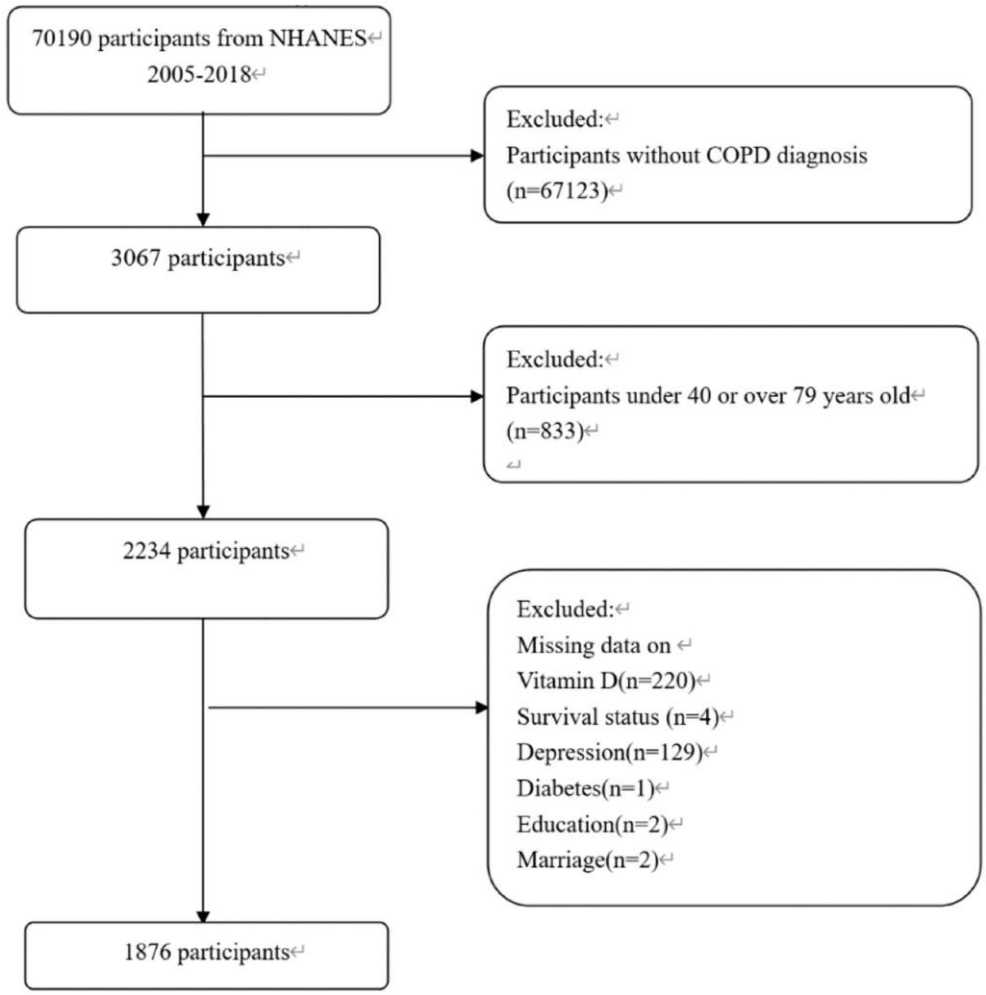

FIGURE 1
Cohort flow diagram in NHANES 2005–2018.

mortality patterns and primary causes of death. Detailed information on mortality files and cause-specific definitions can be found at the CDC Data Link – Mortality Public Information.

25-Hydroxyvitamin D measurements

The serum concentration of 25-hydroxyvitamin D (25(OH)D) serves as the biomarker for assessing vitamin D status. Serum 25(OH)D level classifications follow guidelines from the Endocrine Society Clinical Practice (22). The classifications are as follows: Q1 (<50.0 nmol/L) for deficiency; Q2 (50.0–74.9 nmol/L) for insufficiency; and Q3 (≥75.0 nmol/L) for adequacy.

Covariates

Demographic data collected included age (40–49, 50–59, 60–69, 70–79), gender (male/female), race/ethnicity (non-Hispanic black, non-Hispanic white, Mexican American, other), education level (less than high school, high school equivalent, higher), marital status (married/partner, widowed/divorced/separated, single), and smoking status (never, former, current). "Never smoked" refers to individuals with less than 100 cigarettes in their lifetime, "Former smokers" to those who quit smoking after the same threshold, and "Current smokers" to those still smoking after 100 cigarettes.

Hypertension was identified if participants reported a diagnosis on multiple visits, received prescription recommendations, or had mean systolic ≥140 mm Hg or diastolic ≥90 mm Hg across three measurements. Diabetes mellitus (DM) was confirmed by positive responses to insulin use, physician-diagnosed diabetes, or blood sugar control medication. A CVD history was determined by positive responses to doctor-diagnosed myocardial infarction, angina, coronary heart disease, or stroke. The MetS group included individuals meeting at least three criteria: (1) triglycerides >150 mg/dL; (2) waist circumference ≥ 102 cm for men or ≥ 88 cm for women; (3) HDL levels ≥40 mg/dL for men or ≥ 50 mg/dL for women; (4) blood pressure ≥ 130/≥85 mm Hg; and (5) fasting blood glucose ≥110 mg/dL. Depressive status was assessed using participants' PHQ-9 questionnaire responses. The PHQ-9 is a 9-item self-report depression scale assessing symptom frequency over the past 2 weeks. Items are scored from 0 (none) to 3 (almost daily). PHQ-9 scores

range from 0 to 27, categorized into two groups: "not depressed" for scores <5 and "depressed" for scores of 5 or higher. The DII was calculated based on the 24 h dietary recall data from day one. DII calculation incorporated 26 dietary parameters, including carbohydrates, protein, total fat, saturated fat, PUFA, n-3 fatty acids, cholesterol, energy, alcohol, fiber, folate, iron, magnesium, zinc, selenium, MUFA, caffeine, niacin, riboflavin, thiamine, beta-carotene, and vitamins A, B6, B12, C, and E. Initially, calculate the subjects' average nutrient intake, subtract the global mean, and divide by the standard deviation to obtain Z-scores. Next, Z-scores are converted to percentiles, doubled, and reduced by one to recenter the data. Multiply the central percentile value of each parameter by its inflammatory effect score to calculate a "food-specific DII score." Lastly, values are combined for an "overall DII score" (23–25).

Statistical analysis

To mitigate bias from oversampling, we applied sample weights as per NHANES guidelines. We present normally distributed continuous variables with mean and standard deviation, and categorical variables with frequency and proportion. We used ANOVA for continuous variables and Pearson's chi-square for categorical variables to assess mean differences and proportions. Serum 25-hydroxyvitamin D concentration was treated as a categorical variable. The Kaplan–Meier model assessed cumulative all-cause mortality rates among COPD participants with varying serum 25-hydroxyvitamin D levels. We utilized the Cox proportional hazards model to examine the effect of varying 25-hydroxyvitamin D levels on all-cause mortality in COPD patients. Model 1 featured a univariate analysis of 25-hydroxyvitamin D levels; Model 2 adjusted for age, gender, race/ethnicity, marriage, education, and smoking status. Model 3 additionally adjusted for hypertension, diabetes, cardiovascular disease, metabolic syndrome, and depression, based on Model 2. We investigated a potential non-linear relationship between 25-hydroxyvitamin D and all-cause mortality in COPD patients using a restricted cubic spline (RCS) to assess continuous 25-hydroxyvitamin D levels, with knots at the 5th, 50th, and 95th percentiles. If non-linear, we performed segmented linear regression for further analysis. We conducted a mediation analysis to assess the potential mediating role of vitamin D between DII and mortality. Lastly, subgroup and sensitivity analyses were performed to confirm the results' robustness. All regressions have undergone goodness of fit testing. All analyses were performed using R (version 4.2.0).

Results

Characteristics of participants

Participant demographics and characteristics are presented in Table 1. Participants were predominantly aged 60 or older (50.75%) and women (60.50%). Additionally, 78.51% identified as non-Hispanic white. Those with higher 25-hydroxyvitamin D levels were more likely to be older, non-Hispanic white, married or cohabitating, highly educated, and non-smokers. They also exhibited lower prevalence of CVD, depression, and metabolic syndrome, with no significant differences in diabetes and hypertension rates.

Serum 25(OH)D concentrations and mortality

Over the follow-up period, 395 participants died, with a median duration of 70 months. The Kaplan–Meier curve revealed significantly elevated all-cause mortality rates among COPD patients with lower vitamin D levels ($p = 0.003$) (Figure 2A). The Cox proportional hazards model confirmed increased mortality rates for serum 25(OH)D deficiency categories across all models. In the fully adjusted Model 3, the stratified HRs and 95% CIs for serum 25(OH)D categories were as follows: Q1 (<50.0 nmol/L) as reference, Q2 (50.0–74.9 nmol/L) at 0.63 (0.47–0.83), and Q3 (≥75.0 nmol/L) at 0.52 (0.37–0.72), with a significant decreasing trend (p-trend <0.001) (Table 2). The RCS analysis demonstrated a non-linear association between serum 25(OH)D levels and all-cause mortality in COPD patients (p-nonlinearity = 0.023) (Figure 2B). Additional analyses, including threshold effects and segmented linear regression, were conducted to explore the relationship between serum 25(OH)D levels and all-cause mortality in COPD patients. The findings identified a threshold at 65.3 nmol/L. Above 65.3 nmol/L, serum 25(OH)D levels did not significantly correlate with mortality. Conversely, below 65.3 nmol/L, a negative correlation with mortality was observed across all models (Table 3).

Causal mediation analysis

In order to discover the potential mediating effect of serum vitamin D on inflammatory diet and mortality in COPD patients, and to provide value for improving prognosis, we conducted a mediation analysis. Firstly, the Cox proportional hazards model shows a positive correlation between DII and all-cause mortality in the COPD population (Supplementary Table S1). Secondly, correlation analysis shows a negative correlation between DII and serum vitamin D levels (Supplementary Table S2). Finally, the mediation analysis results showed a mediation effect of 22% (95% CI, 0.07–0.74) (Table 4), confirming our hypothesis.

Subgroup and sensitivity analysis

We performed subgroup analyses to assess the influence of demographic factors and comorbidities on the association between serum 25(OH)D concentration and all-cause mortality in patients with COPD. The findings indicated no significant interactions in stratified analyses by sex, marriage, education, smoking status, diabetes, hypertension, cardiovascular disease, metabolic syndrome, and depression ($p > 0.05$). However, significant interactions were observed between serum 25(OH)D concentration and both age and race/ethnicity (Figure 3). Sensitivity analysis, excluding participants with less than 2 years of follow-up and considering 25(OH)D as a continuous variable, produced consistent results (Supplementary Tables S3, S4), confirming the robustness of our findings.

Discussion

Our study utilized the NHANES mortality cohort data from 2005 to 2018 to assess the relationship between serum 25(OH)D

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TABLE 1 Baseline characteristics of participants with COPD according to serum 25(OH)D concentrations.

Characteristics	Serum 25(OH)D Concentrations (nmol/L)				
	Total	<50 (*n* = 592)	50–74.9 (*n* = 615)	> = 75.0 (*n* = 669)	*p*-value
Age, years					**0.004**
40–49	336 (20.83)	127 (25.23)	91 (17.30)	118 (21.99)	
50–59	472 (28.42)	153 (31.04)	155 (24.42)	164 (31.54)	
60–69	618 (31.60)	186 (25.79)	232 (35.41)	200 (31.16)	
70–79	450 (19.15)	126 (17.94)	191 (22.88)	133 (15.31)	
Gender, %					0.09
Female	1,070 (60.50)	344 (62.73)	394 (62.66)	332 (56.04)	
Male	806 (39.50)	248 (37.27)	275 (37.34)	283 (43.96)	
Race/ethnicity, %					**<0.0001**
White	1,088 (78.51)	254 (66.30)	471 (86.11)	363 (78.12)	
Black	378 (8.82)	204 (19.27)	80 (4.27)	94 (6.66)	
Mexican American	126 (2.51)	49 (3.71)	37 (1.98)	40 (2.29)	
Others	284 (10.16)	85 (10.73)	81 (7.65)	118 (12.93)	
Marriage, %					**<0.0001**
Married/Living with partner	959 (58.94)	265 (48.48)	375 (67.27)	319 (56.25)	
Widowed/divorced/separated	740 (33.64)	258 (40.33)	240 (27.30)	242 (36.66)	
Never married	177 (7.42)	69 (11.18)	54 (5.43)	54 (7.09)	
Education, %					
<High school	524 (19.22)	199 (25.67)	140 (13.14)	185 (22.07)	**<0.0001**
High school	492 (28.86)	156 (30.84)	192 (29.40)	144 (26.66)	
>High school	860 (51.92)	237 (43.49)	337 (57.46)	286 (51.26)	
Smoking status, %					**0.002**
Never	496 (27.37)	153 (26.71)	179 (29.43)	164 (25.23)	
Former	687 (35.97)	183 (27.67)	287 (41.22)	217 (35.60)	
Now	693 (36.66)	256 (45.61)	203 (29.35)	234 (39.17)	
DM, %					0.98
Yes	569 (25.54)	189 (25.83)	213 (25.68)	167 (25.13)	
No	1,307 (74.46)	403 (74.17)	456 (74.32)	448 (74.87)	
Hypertension, %					0.15
Yes	1,230 (59.94)	397 (65.37)	446 (57.79)	387 (58.53)	
No	646 (40.06)	195 (34.63)	223 (42.21)	228 (41.47)	
Cardiovascular disease, %					**0.002**
Yes	611 (28.58)	211 (36.92)	203 (23.65)	197 (28.51)	
No	1,265 (71.42)	381 (63.08)	466 (76.35)	418 (71.49)	
Depression, %					**0.001**
Yes	863 (41.31)	295 (50.52)	278 (34.81)	290 (42.60)	
No	1,013 (58.69)	297 (49.48)	391 (65.19)	325 (57.40)	
Metabolic syndrome, %					**0.01**
Yes	1,018 (51.59)	338 (59.32)	363 (48.29)	317 (49.92)	
No	858 (48.41)	254 (40.68)	306 (51.71)	298 (50.08)	
Serum 25(OH)D Concentrations, nmol/L	71.49 (1.44)	35.28 (0.52)	62.95 (0.33)	99.79 (1.60)	**<0.0001**
Dietary Inflammatory Index	1.84 (0.07)	2.27 (0.10)	1.52 (0.09)	1.92 (0.11)	**<0.0001**

Bold values (when *p* is less then 0.05) means it is statistically significant.

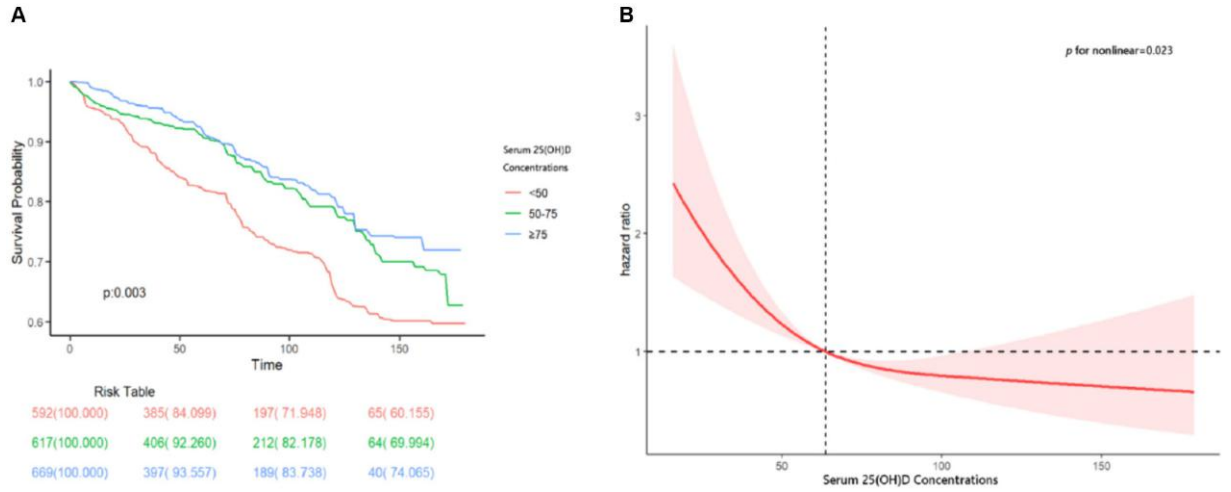

FIGURE 2
(A) The cumulative incidence of all-cause death in the three groups of serum 25(OH)D concentrations during the follow-up period. **(B)** The restricted cubic spline (RCS) analyses between serum 25(OH)D and all-cause mortality of participants with COPD. RCS adjusted for age, gender, race/ethnicity, marriage, education, smoking status, hypertension, diabetes, cardiovascular disease, metabolic syndrome, and depression.

TABLE 2 **The relationship between serum 25(OH)D concentrations and all-cause mortality of COPD among participants from the NHANES (2005–2018).**

Models	Serum 25(OH)D Concentrations (nmol/L), HR (95% CI)					
	Model 1		Model 2		Model 3	
Character	95% CI	*p*	95% CI	*p*	95% CI	*p*
<50	Ref		Ref		Ref	
50–74.9	0.63 (0.45,0.89)	**0.01**	0.56 (0.43, 0.73)	**<0.0001**	0.63 (0.47, 0.83)	**0.001**
>=75	0.53 (0.37,0.76)	**<0.001**	0.44 (0.32, 0.62)	**<0.0001**	0.52 (0.37, 0.72)	**<0.001**
p for trend		**<0.001**		**<0.0001**		**<0.001**

Model 1: non-adjusted.
Model 2: adjusted for age, gender, race/ethnicity, marriage, education, and smoking status.
Model 3: adjusted for model 2 plus hypertension, diabetes, cardiovascular disease, metabolic syndrome, and depression.
Bold values (when *p* is less then 0.05) means it is statistically significant.

TABLE 3 **Threshold effect analysis of serum 25(OH)D concentrations on all-cause mortality in COPD patients.**

	Adjusted HR (95% CI), *p*-value
Fitting by the standard linear model	0.99 (0.98, 1.00) < 0.001
Fitting by the two-piecewise linear model	
Inflection point	65.30 nmol/L
25(OH)D concentrations <65.30 nmol/L	0.98 (0.97, 0.99) 0.005
25(OH)D concentrations >= 65.30 nmol/L	0.99 (0.98, 1.00) 0.21

Adjusted for age, gender, race/ethnicity, marriage, education, smoking status, hypertension, diabetes, cardiovascular disease, metabolic syndrome, and depression.

concentrations and all-cause mortality among US COPD patients aged 40 to 79. First, our findings indicate that 64.34% of COPD patients have serum 25(OH)D deficiency, underscoring a widespread prevalence of insufficient vitamin D levels consistent with previous research (26–28). Second, an L-shaped correlation between serum 25(OH)D levels and all-cause mortality was observed in COPD patients, suggesting that, within a specific range, reduced levels significantly associate with increased all-cause mortality. The observed association remains significant, independent of conventional lifestyle factors and prevalent comorbidities such as diabetes, hypertension, cardiovascular disease, and metabolic syndrome, with subgroup analysis corroborating this conclusion. These results may lead to clinical and dietary guidelines appears.

In individuals with COPD, two key factors contributing to breathing difficulties and restricted airflow are inflammation within the bronchial tube linings (obstructive bronchiolitis) and the destruction of alveolar sacs (emphysema) (29). Obstructive bronchiolitis is characterized by mucus cell hyperplasia, an increase in smooth muscle cells, and fibrosis in the airways (30). The development of emphysema is attributed to an imbalance in protease/

TABLE 4 Mediation effects of 25(OH)D on association of DII and all-cause mortality.

Independent variable	Mediator	Total effect		Indirect effect		Direct effect		Proportion mediated, % (95% CI)
		Coefficient (95% CI)	*p* value	Coefficient (95% CI)	*p* value	Coefficient (95% CI)	*p* value	
DII	25(OH)D	−35.48 (−71.12, −6.93)	0.016	−7.55 (−14.63, −2.55)	<0.001	−27.93 (−63.61, −1.23)	0.028	0.22 (0.07, 0.74)

Mediation analysis was adjusted for age, gender, race/ethnicity, marriage, education, smoking status, hypertension, diabetes, cardiovascular disease, metabolic syndrome, and depression.

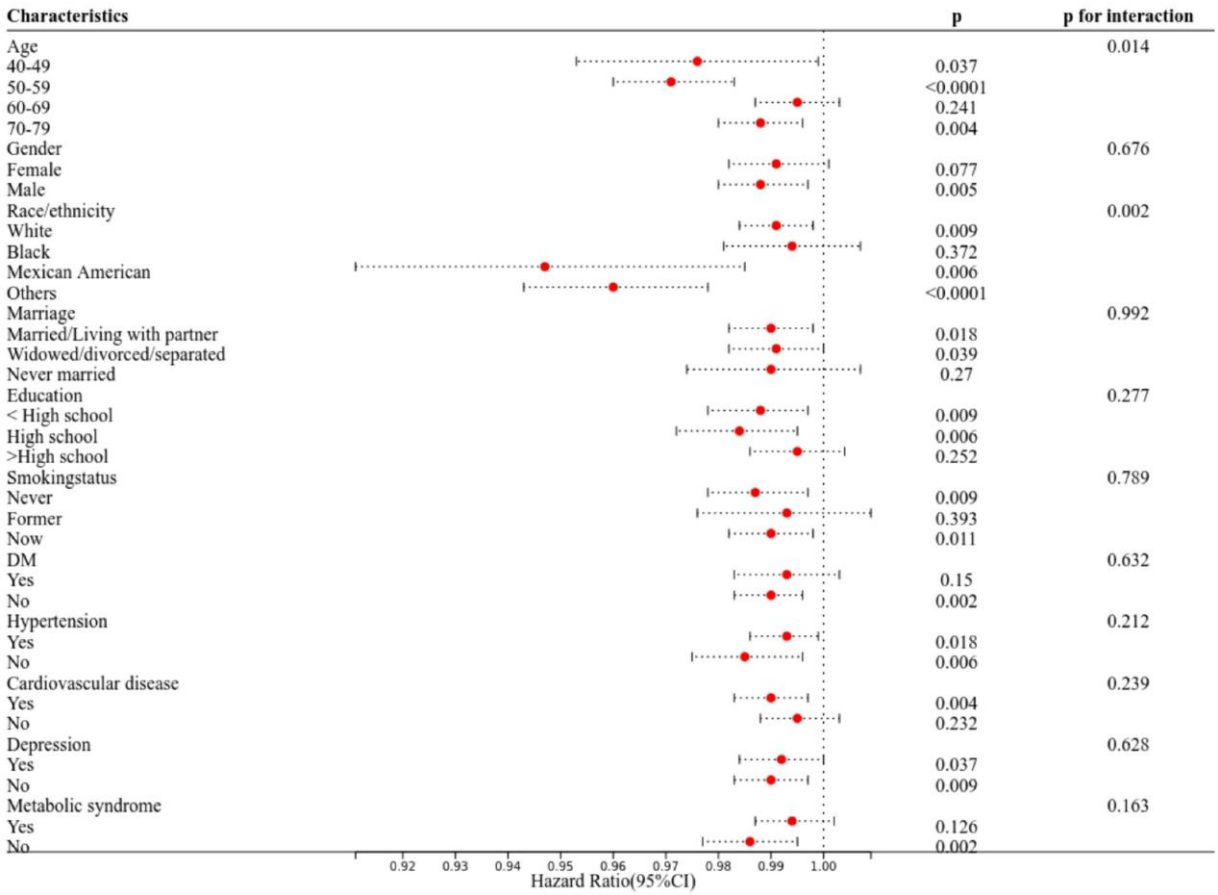

FIGURE 3
Subgroup analyses of the associations between serum 25(OH)D concentrations and all-cause mortality among participants with COPD from the NHANES (2005–2018). The Cox proportional hazard model was used to estimate the HR of all-cause mortality.

antiprotease enzyme activity (31). Prolonged chronic inflammation can lead to the generation of endogenous reactive oxygen species (ROS), resulting in an imbalance in oxidants/antioxidants (32). Furthermore, ROS can activate various pro-inflammatory pathways, such as nuclear factor kB (NF k β) and the MAPK pathway, thereby triggering inflammation (33). Studies have indicated a decrease in Nrf2 gene expression in COPD patients, which may contribute to oxidative stress in lung tissue (34). Insufficient levels of vitamin D have been associated with the progression of various pathological processes in COPD. These processes include the regulation of inflammation, heightened oxidative stress, increased expression of proteases, compromised host defense, and remodeling of the pulmonary airway (10). According to a study a deficiency in vitamin D can lead to an increase in the production of several matrix metalloproteinases (MMP2, MMP9, and MMP12), resulting in an imbalance in protease/antiprotease expression (35). Additionally, vitamin D inhibits the TGF-b1 signaling pathway, which is linked to fibrosis in COPD (36). Numerous studies have highlighted the potential antioxidant properties of vitamin D and its analogues, as well as their ability to activate Nrf2 (37). Vitamin D stimulates Nrf2 expression, thereby enhancing the phagocytic potential of alveolar macrophages in COPD patients. Chronic inflammation and oxidative stress play a crucial role in the development of COPD, the related function of vitamin D could potentially serve as an effective therapeutic target.

Several prospective cohort studies suggest an association between vitamin D deficiency and diminished lung function or an

increased risk of acute exacerbation in COPD (18, 38, 39). Furthermore, multiple randomized controlled trials (RCTs) have demonstrated that vitamin D supplementation can decrease the incidence of moderate or severe COPD exacerbations in patients with lower baseline concentrations of 25(OH)D (13, 40). Additionally, a meta-analysis incorporating vitamin D and protein gene polymorphism studies has highlighted a connection between vitamin D status and COPD risk (41). Although vitamin D is recognized for its crucial role in bone health and various chronic diseases, the optimal serum 25(OH)D concentration remains contentious. Our findings parallel those of other diseases, specifically the link between low serum 25(OH)D concentrations and all-cause mortality. This relationship is typically non-linear, with mortality rates diminishing as 25(OH)D levels rise up to a threshold point, beyond which no further reduction occurs. Compared with other similar studies, our study has more standardized and high-quality data sources and a longer follow-up time, we also identified a 63.40 nmol/L threshold for all-cause mortality in COPD patients, however, confirming whether serum 25(OH)D concentrations at or above this level mitigate the risk of premature death requires further clinical trials.

Current research suggests that dietary inflammation is associated with the incidence of COPD, deterioration in lung function, and disease progression, potentially linked to the chronic inflammatory nature of such diets contributing to COPD's progression (42). However, the relationship between diet-related inflammation and COPD mortality remains poorly understood, and it is unclear whether vitamin D deficiency in COPD patients is related to this phenomenon or not. Our study utilized the Dietary Inflammatory Index (DII) to quantify dietary inflammation. DII is designed based on the influence of dietary parameters on inflammatory biomarkers (IL-4, IL-6, IL-10, TNF-α, and CRP), which may stimulate the activation of CYP27B1 (43). CYP27B1 is an enzyme that converts 25 (OH) D into its active form 1,25 $(OH)_2$ D. Elevated 1,25 $(OH)_2$D can inhibit the conversion of vitamin D_3 to 25 (OH)D and the liver synthesis of 25(OH)D, consequently resulting in a reduction in serum 25(OH) D levels (44). Findings indicated a positive correlation between DII and COPD mortality, a negative correlation with serum 25(OH)D level. Additional mediation analysis supported serum 25(OH)D's mediating role between dietary inflammation and mortality. Different from other studies, this new discovery in COPD patients could potentially establish a novel pathway hypothesis, leading to new treatment avenues in the future.

This study has several notable strengths. Initially, the study included a nationally representative sample of American adults with COPD, which ensured the generalizability of the results thanks to the large sample size. Additionally, the extended follow-up period for tracking fatalities provides a robust foundation for the study's analysis. Secondly, meticulous adjustments for socioeconomic status, dietary and lifestyle factors, comorbidities, and other potential confounders strengthen our conclusions. Finally, using standardized methods to ascertain serum 25(OH)D concentrations in the NHANES database ensures the reliability of our data analysis.

However, this research has its limitations. First, the observational nature of the study does not allow for the establishment of causation. Second, a single 25(OH)D measurement at recruitment may not accurately capture long-term exposure levels. Nonetheless, other studies indicate that a single measurement can adequately reflect vitamin D status over time (45), and a moderate ICC suggests that time-dependent variation is unlikely to significantly affect the study's findings. Third, the inclusion of COPD patients was based on initial questions without subsequent verification from medical records. Lastly, like other observational studies, this research cannot rule out the possibility of residual or unknown confounding, or unanticipated confounding effects due to measurement errors and unmeasured variables.

In summary, considering multiple factors, this study discovered a significant and consistent association between lower serum 25(OH)D levels and increased risks of death from all causes, as well as its mediating role in the impact of the Dietary Inflammatory Index on mortality among American adults with COPD. This finding could serve as a target for interventions aimed at decreasing the risk of premature death. The findings underscore the importance of monitoring and evaluating vitamin D levels to prevent mortality in adults with COPD and provide a possible preventive approach.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: NHANES.

Ethics statement

The studies involving humans were approved by the National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

QJ: Conceptualization, Data curation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. YJ: Conceptualization, Formal analysis, Supervision, Writing – review & editing. ZM: Conceptualization, Software, Validation, Writing – review & editing. JH: Conceptualization, Methodology, Writing – review & editing. YL: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1412606/full#supplementary-material

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The effects of vitamin D supplementation on serum lipid profiles in people with type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials

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Introduction: People with type 2 diabetes (T2D) are highly susceptible to the development of cardiovascular diseases. Previous studies have suggested that the application of vitamin D may offer potential benefits in improving lipid profiles, but these effects remain controversial.

Methods: This systematic review and meta-analysis focused on the effects of vitamin D supplementation on serum lipid profiles in people with T2D. Randomized controlled trials (RCTs) assessing the effects of vitamin D supplementation on lipid profiles and published before September 19th, 2023, were identified in PubMed, Embase, and Cochrane Library. This review protocol was registered in the PROSPERO (CRD42023461136). The random-effects model was employed to estimate unstandardized mean differences (MD) and 95% confidence intervals (CIs). The quality of studies was assessed by the Cochrane Risk of Bias tool 2.

Results: Overall, 20 RCTs involving 1711 participants were included. Results indicated that vitamin D supplementation significantly improves serum high-density lipoprotein (HDL) (MD: 1.63 mg/dL, 95% CI: 0.19 to 3.08, P = 0.03), and triglyceride (TG) levels (MD: -8.56 mg/dL, 95% CI: -15.23 to -1.89, P = 0.01). However, vitamin D supplementation failed to improve low-density lipoprotein (LDL) levels and total cholesterol (TC) levels. Subgroup analyses and meta-regressions suggested that higher doses of vitamin D supplementation and shorter duration of intervention were more likely to have favorable effects on lipid profiles. Moreover, participants with lower baseline BMI and higher serum 25-hydroxy vitamin D levels exhibited greater improvements in lipid profiles following vitamin D supplementation.

Conclusions: This meta-analysis highlighted the effects of vitamin D supplementation on improving serum HDL and TG levels while not exhibiting significant improvements in LDL and TC levels. Further long-term and high-quality studies are still needed to draw more precise conclusions.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=461136.

KEYWORDS

type 2 diabetes, vitamin D supplementation, lipid profiles, meta-analysis, cardiovascular diseases

1 Introduction

Type 2 Diabetes (T2D) has emerged as a significant public health concern globally, with its prevalence expected to rise from 536.6 million in 2021 to 780 million by 2045 (1). People with T2D are at an elevated risk of developing cardiovascular diseases (CVD). The incidence of CVD among people with T2D is 2 to 3 times higher compared to non-diabetic persons (2). Dyslipidemia is frequently observed in people with T2D, which is also a widely recognized risk factor for CVD. It is characterized by raised levels of serum total cholesterol (TC) and low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) levels (3).

Vitamin D is a fat-soluble vitamin that naturally occurs in a limited number of foods and can be acquired through dietary supplementation (4). It is essential for maintaining bone health and has additional benefits for extra-skeletal effects such as regulation of inflammation (4, 5). Auto-immune activation and low-grade inflammation play significant roles in the onset and progression of T2D, as increased inflammatory cytokine activity would cause beta-cell death in the pancreas and raise insulin resistance in target cells (6). Similarly, vitamin D may lower the risks of CVD via the mechanism of down-regulating inflammation and increase insulin sensitivity (7), indicating its potential to prevent both T2D and CVD.

Vitamin D status is determined by serum 25-hydroxy vitamin D (25OHD) concentration. The Endocrine Society defines vitamin D status based on serum levels of 25OHD, with deficiency indicated by less than 50 nmol/L (8, 9). People with T2D may have lower serum 25OHD levels and thus be more susceptible to vitamin D deficiency (10). Moreover, deficient vitamin D levels may also be associated with unfavorable serum lipid profiles, particularly TC, LDL and HDL levels (11).

The effects of vitamin D on lipid profiles among people with T2D have been investigated in these two decades. A previous meta-analysis conducted by Jafari et al. (6) in 2016 demonstrated a significant reduction in serum LDL and TC levels with the administration of vitamin D, while no significant effect was observed on triglyceride (TG) levels. The effects on serum HDL levels were statistically significant but the absolute difference was negligible (6). Through their subgroup analyses, only doses of vitamin D less than 2000 IU significantly decrease TG and TC levels, while only interventions lasting less than 12 weeks significantly reduce LDL and HDL levels (6). However, recent randomized controlled trials (RCTs) have produced inconsistent results compared to the findings of the previous meta-analysis. Hu et al. (12) revealed no significant change in LDL, HDL and triglycerides (TG) levels by supplementation with 800 IU vitamin D per day for 30 months. Nevertheless, El Hajj et al. (13) demonstrated that administration of 4,000 IU/day of vitamin D, for 6 months, significantly reduced TG level and increased HDL level, but insignificantly changed levels of LDL and TC. Therefore, further consensus regarding the impact of vitamin D supplementation on lipid profiles remains to be established.

In addition, the meta-analysis conducted by Jafari et al. (6) also pointed out that vitamin D fortification may yield more favorable effects on lipid profiles compared to supplementation. Given that vitamin D fortification often employs lower doses and shorter intervention periods (14, 15), the effects of vitamin D (fortification and supplementation) on lipid profiles may differ compared to solely administering vitamin D supplementation. However, to our knowledge, there is currently no existing meta-analysis purely focused on investigating the impact of vitamin D supplementation on lipid profiles among individuals with T2D. As such, we performed a systematic review and meta-analysis specifically focused on vitamin D supplementation to examine its effects on lipid profiles (TC, TG, LDL, and HDL) in people with type 2 diabetes. Our secondary outcomes involve examining the influence of variables such as dose, duration, baseline 25 OHD levels, BMI, type of vitamin D supplementation, and publication year on the effects of vitamin D supplementation on lipid profiles.

2 Materials and methods

The protocol has been registered in the international Prospective Register of Systematic Reviews (PROSPERO, registration number: CRD42023461136). This review was performed based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (16).

2.1 Literature search strategies

Three databases including PubMed, Embase, and Cochrane Library were searched. RCTs were selected from database inception up to September 19th, 2023. The search terms including Medical subject headings (MeSH) and non-MeSH search terms were presented as follows: ((“Vitamin D” OR “ergocalciferols” OR “Vitamin D2” OR “ergocalciferol” OR “25-hydroxyvitamin D2” OR “Dihydrotachysterol” OR “calcifediol” OR “cholecalciferol” OR “Hydroxycholecalciferols” OR “Calcitriol” OR “dihydroxycholecalciferol” OR “Calciferol”) AND (“Type 2 Diabetes Mellitus” OR “Type 2 Diabetes” OR “Noninsulin Dependent Diabetes Mellitus” OR “Diabetes, Type 2” OR “Diabetes Mellitus” OR “type 2 diabetes” OR “type 2 diabetes mellitus”) AND (“Intervention” OR “controlled trial” OR “randomized” OR “randomised” OR “placebo” OR “trial” OR “Trial” OR “trials” OR “randomized controlled trial” OR “randomised controlled trial” OR “RCT” OR “blinded” OR “double blind” OR “double blinded” OR “clinical trial” OR “Cross-Over” OR “parallel” OR “randomly”)). Moreover, we manually search references to avoid missing additional eligible studies.

2.2 Study selection

Two researchers (Qingyang Lu and Yue Xi) independently ascertained eligible studies by reading titles, abstracts, and full text if necessary. The inclusion studies were those written in English only. We only included parallel RCTs that provided sufficient information to examine the effects of vitamin D supplementation on lipid profiles LDL, HDL, TC, and TG in people with T2D. All types of vitamin D such as vitamin D3 (cholecalciferol), vitamin D2 (ergocalciferol), calcitriol (1, 25-hydroxyvitaminD3), and unspecified types of vitamin D treated in intervention were included. We excluded studies if: (i) studies were duplicated, conference papers, letters, reviews, animal studies, observational studies, RCTs with inappropriate control or intervention groups, or open-label RCTs; (ii) participants of studies were pre-diabetes, type 1 diabetes mellitus, gestational diabetes

mellitus, or T2D with nephropathy; (iii) studies lacked adequate data in terms of lipid profiles and baseline information; (iv) studies used vitamin D fortification. Any discrepancies in the study selection process were discussed with a third researcher (Qingyue Liang).

2.3 Data extraction and quality assessment

We extracted data from each study including: (i) basic characteristics of studies regarding first author's last name, publication year, study location, intervention duration, doses of vitamin D supplementation and sample size in each arm. Dose were uniformly calculated as daily dose (international unit, IU) and the longest duration of intervention was collected; (ii) basic characteristics of participants including mean age, baseline mean serum 25OHD (nmol/L), baseline body mass index (BMI) in each arm; (iii) mean and Standard Deviation (SD) of lipid profiles (at baseline and end of intervention, changes from baseline to end of intervention in each group, or changes from baseline to end of intervention between two groups). The process of data extraction was conducted by two researchers independently (Yue Xi and Qingyang Lu). We followed Cochrane guidelines and used the Cochrane Risk of Bias tool-2 (RoB 2) to assess the quality of each included RCT (17, 18). The ROB 2 tool comprises five domains and assigns judgments of "Low," "Some concerns," and "High" within each domain (18).

2.4 Data synthesis

Data of lipid profiles reported in millimoles per liter were manually converted into milligrams per deciliter by multiplying with 38.67 for LDL, HDL, and TC, and by 88.57 for TG (19). For data given adjusted coefficients and 95% confidence intervals (CI), CI was converted into SDs by using the formula $\sqrt{n} \times \frac{(UL - LL)}{3.92}$ if the sample size is over 50. For data presented as median and the first and the third quartiles, the mean was calculated based on the method proposed by Luo et al. (20) and SD was estimated based on the findings of Wan et al. (21). If the change of lipid profiles were not given directly in the original studies, the mean of changes was calculated by subtracting the baseline from end-of intervention in each group. Related SD was imputed by using $\sqrt{SD_b^2 + SD_f^2 - \left(2 \times 0.5 \times SD_b \times SD_f\right)}$, where SD_b was baseline SD and SD_f was end-of-intervention SD and the correlation coefficient was assumed as 0.5 (22).

2.5 Statistical analysis

The unstandardized mean difference (MD) and 95% confidence interval (CI) between the control and intervention groups were estimated using random-effects models in this meta-analysis (23). Heterogeneity between studies was estimated by Cochran's Q test, Tau-squared (tau^2) and I-squared (I^2) value. The values of I^2 below or equal to 25%, between 26 and 50%, and above 50% are denoted as low, moderate, and high heterogeneity, respectively (6). To identify resources of heterogeneity and mean differences among various factors, subgroup analyses were conducted based on baseline 25 OHD level (≥ 50 nmol/L and < 50 nmol/L), baseline BMI (≥ 30 kg/m^2 and < 30 kg/m^2), vitamin D doses (≥ 5,000 IU/day and < 5,000 IU/day), intervention duration (≥ 24 weeks and < 24 weeks), publication year (2016 and before, 2017 and beyond) and type of vitamin D supplementation. Moreover, meta-regression analyses were applied if variables were continuous, such as doses, duration, baseline 25 OHD and BMI. Permutation tests were additionally conducted to ensure the robustness of meta-regressions. Permutation resampling is applied to assess the absence of an effect in scenarios where there is uncertainty regarding the distribution of the test statistic or when the data are not randomly sampled from a defined population (24). We also performed the leave-one-out analysis as a sensitivity analysis of heterogeneity. In addition, publication bias was detected by using the contour-enhanced funnel plot, Egger's test, and Begg's test. The plots of quality assessments were conducted by using the R package "robvis" (25). The statistical analysis was conducted by using R software, Version 4.3.0. All statistical tests were performed as two-sided, and the significance level was set at $p < 0.05$.

3 Results

3.1 Study selection

The process of study selection and identification is presented in Figure 1. In total, 2,603 articles were found in database searching, out of which 927 were identified as duplicates. The remaining 1,676 articles were screened by title and abstract, with 1,588 articles excluded due to irrelevance to the topic or other reasons. Eighty-eight articles were assessed for eligibility, and an additional 68 studies were excluded due to the following reasons: lacked information on lipid profiles, usage or doses of vitamin D supplementation, baseline 25OHD or BMI, or administration of vitamin D fortification ($n = 47$); inappropriate control or intervention group ($n = 20$); not mention the specific type of diabetes ($n = 1$). Finally, 20 articles with 24 effect sizes were included (12, 26–44).

3.2 Study characteristics

Detailed characteristics of included studies are presented in Table 1. Overall, most of the studies (n = 13) were conducted in Asia (12, 26, 29, 30, 32, 33, 35, 37–39, 41, 42, 44). Thirteen studies clarified using vitamin D3 as their supplementation (12, 26–32, 34–36, 40, 43), while the remaining 7 studies did not specify the type of vitamin D (33, 37–39, 41, 42, 44). A total of 1711 participants were admitted into these 20 studies, with 861 participants in the intervention group and 851 participants in the control group. At baseline, the mean age of all participants was 54.9 years (range: 42.3 to 66.3 years), the average BMI was 29.6 kg/m2 (range: 24.7 to 37.8 kg/m2) and the average level of 25 OHD was 46.6 nmol/L (range: 23.6 to 86.8 nmol/L). The duration of the intervention ranged from 8 to 120 weeks, with an average of 22.2 weeks, while the dosage of vitamin D supplementation varied from 800 to 7142.9 IU/day, with a mean of 4341.27 IU/day. The quality assessments indicated that most of the included studies had low quality or were discovered with some concerns, except of the study conducted by Al-Zahrani et al. (26), which was deemed to have high risks. The detailed results of the quality assessment are presented in Figure 2.

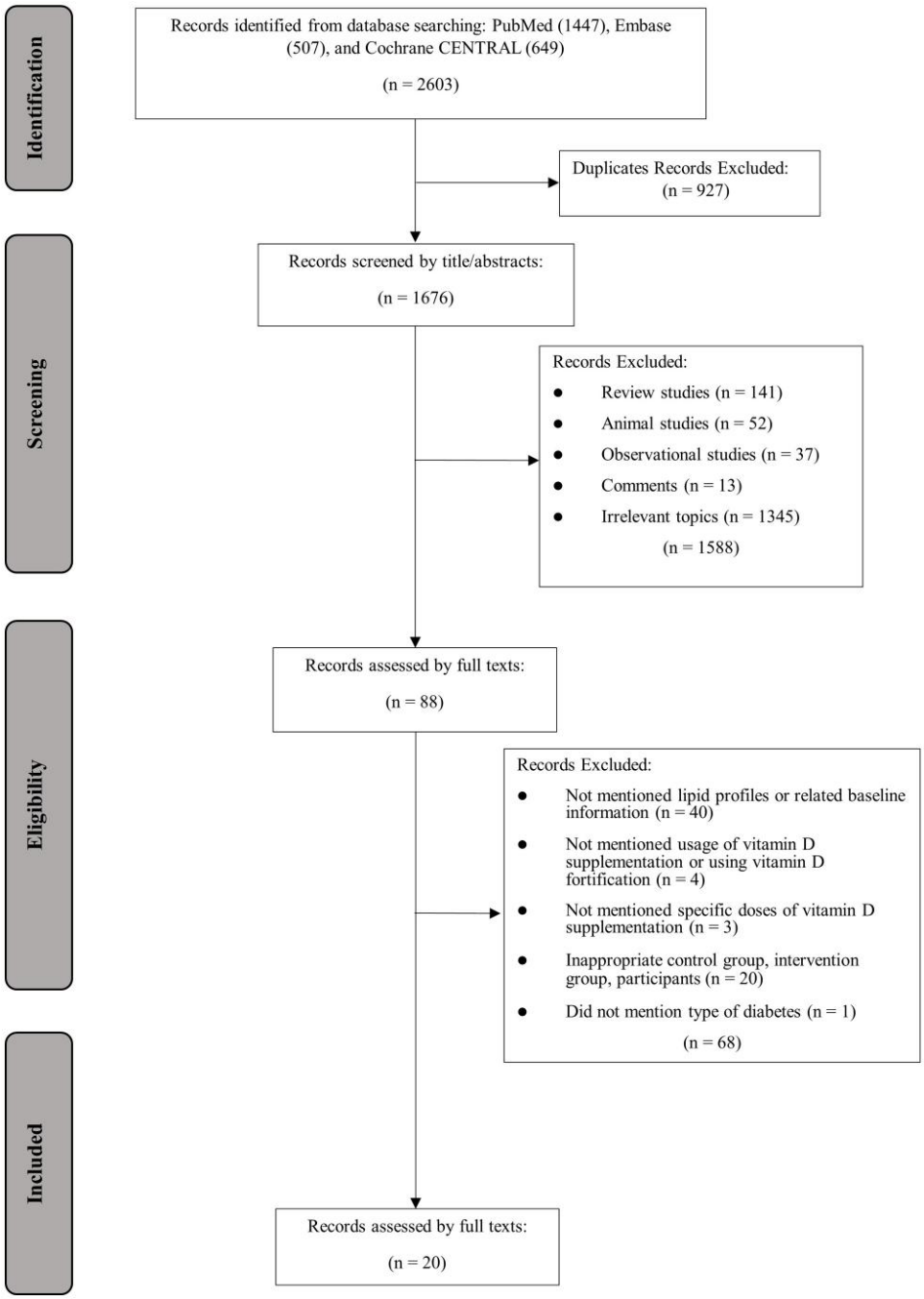

FIGURE 1
The PRISMA flowchart of study search, screening, selection, and inclusion.

3.3 Effect of vitamin D supplementation on serum LDL level

A total of 19 studies with 22 effect sizes (1,633 participants included) reported the data regarding LDL levels. This meta-analysis did not demonstrate any significant effects of vitamin D supplementation on LDL levels (−1.33 mg/dL, 95% CI: −4.71 to 2.05, $p = 0.44$; $I^2 = 84.6\%$, $p < 0.01$, Figure 3). Nevertheless, the subgroup analyses revealed a significant difference in the effects of vitamin D supplementation among different dosage groups, with a cutoff of 5,000 IU ($p = 0.03$). In studies where daily doses exceeded 5,000 IU, a significant reduction in LDL levels was observed (−4.68 mg/dL, 95% CI: −9.15 to −0.21, $p = 0.04$, Figure 4). Meta-regression analyses revealed a potential association between doses and changes in LDL (estimate: -0.0017, 95% CI: −0.0031 to −0.0004, $p = 0.01$, Figure 5; Supplementary Table S1). A similar finding was observed between dose and effect sizes of LDL when implementing permutation tests (estimate: -0.0017, 95% CI: −0.0032 to −0.0002, $p = 0.05$, Supplementary Table S2).

TABLE 1 Baseline characteristics of included RCTs in the meta-analysis.

Study	Country	Type of vitamin D	Sample size		Doses (IU/day)	Duration (week)	Baseline information of participants			Intervention	
			IG	CG			Age (years)	BMI (kg/m^2)	25 OHD (nmol/L)	IG	CG
Jorde et al. (27)	Norway	Vitamin D3	16	16	5714.29	24	56.25	32.05	59.25	Vitamin D	Placebo
Witham et al. (28)	United Kingdom	Vitamin D3	19	21	833.33	16	66.04	32.26	43.10	Vitamin D	Placebo
Witham et al. (28)	United Kingdom	Vitamin D3	18	21	1666.67	16	65.13	31.64	46.38	Vitamin D	Placebo
Breslavsky et al. (29)	Israel	Vitamin D3	24	23	1000.00	48	66.31	28.73	29.69	Vitamin D	Placebo
Yiu et al. (30)	China	Vitamin D3	50	50	5000.00	12	65.35	25.45	53.75	Vitamin D	Placebo
Al-Zahrani et al. (26)	Saudi Arabia	Vitamin D3	91	92	4821.43	12	54.69	31.65	23.64	Vitamin D	No treatment
Kampmann et al. (31)	Denmark	Vitamin D3	7	8	6533.33	12	59.15	33.75	33.03	Vitamin D	Placebo
Ryu et al. (32)	South Korea	Vitamin D3	32	30	2000.00	24	55.56	24.84	28.81	Vitamin D + calcium	Placebo + calcium
Yousefi Rad et al. (33)	Iran	Not specified	28	30	4000.00	8	49.96	28.36	37.70	Vitamin D	Placebo
Muñoz-Aguirre et al. (34)	Mexico	Vitamin D3	52	52	4000.00	24	56.75	30.65	54.55	Vitamin D	Placebo
Sadiya et al. (35)	Ajman	Vitamin D3	43	39	4500.00	24	48.52	37.76	29.45	Vitamin D	Placebo
Barchetta et al. (36)	Italy	Vitamin D3	26	29	2000.00	24	58.67	30.09	43.93	Vitamin D	Placebo
Safarpour et al. (37)	Iran	Not specified	43	42	7142.86	8	50.20	30.89	43.50	Vitamin D	Placebo
Upreti et al. (38)	India	Not specified	30	30	3750.00	24	49.10	25.07	25.99	Vitamin D	Placebo
Dadrass et al. (39)	Iran	Not specified	12	12	3571.43	12	54.33	28.47	35.88	Vitamin D + resistance training	Resistance training
Dadrass et al. (39)	Iran	Not specified	12	12	3571.43	12	53.50	27.75	37.88	Vitamin D	Placebo
Angellotti et al. (40)	United States	Vitamin D3	59	61	4000.00	48	60.20	30.95	66.50	Vitamin D	Placebo
Mirzavandi et al. (41)	Iran	Not specified	25	25	7142.86	8	45.70	29.91	40.00	Vitamin D	No treatment
Hoseini et al. (42)	Iran	Not specified	10	10	7142.86	8	47.73	29.48	54.59	Vitamin D + aerobic training	Aerobic training
Hoseini et al. (42)	Iran	Not specified	10	10	7142.86	8	48.69	28.74	58.09	Vitamin D	Placebo
Derosa et al. (43)	Italy	Vitamin D3	119	113	3571.43	24	54.47	27.52	45.99	Vitamin D	Placebo
Hu et al. (12)	China	Vitamin D3	115	105	800.00	120	66.08	24.71	57.05	Vitamin D	No treatment
Salarinia et al. (44)	Iran	Not specified	10	10	7142.86	8	42.30	29.90	86.75	Vitamin D + water exercise	Water exercise
Salarinia et al. (44)	Iran	Not specified	10	10	7142.86	8	43.30	29.94	84.38	Vitamin D	No treatment

25 OHD: 25-hydroxyvitamin D; IG: intervention group; CG: control group.

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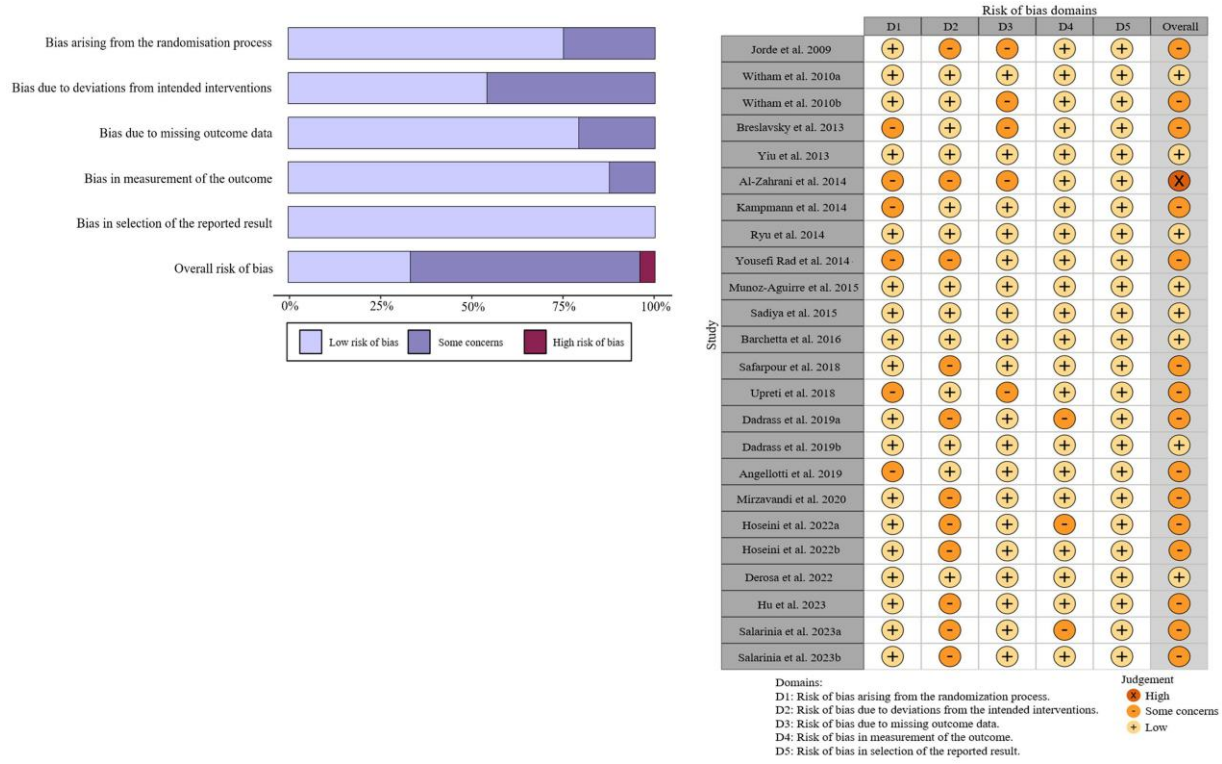

FIGURE 2
Summary **(A)** and graph **(B)** of the risk of bias in the selected studies by the Cochrane Risk of Bias tool-2 (ROB2).

3.4 Effect of vitamin D supplementation on serum HDL level

Nineteen studies with 22 effect sizes (1,633 participants) examined the effects of vitamin D supplementation on HDL levels. Overall, vitamin D supplementation significantly increased HDL (1.63 mg/dL, 95% CI: 0.19 to 3.08, $p = 0.03$; $I^2 = 89.8\%$, $p < 0.01$, Figure 6). For subgroup analyses (Figure 7), studies that did not specify the type of vitamin D supplementation significantly increased HDL (3.92 mg/dL, 95% CI: 1.77 to 6.06, $p < 0.01$). A significant increase in HDL was also observed in studies published beyond 2017 (2.71 mg/dL, 95% CI: 0.80 to 4.62, $p = 0.01$). Furthermore, doses over 5,000 IU per day significantly increased HDL by 3.11 mg/dL (95% CI: 0.69 to 5.53, $p = 0.01$) and trials with a duration (< 24 weeks) showed a significant increase in HDL by 2.20 mg/dL (95% CI: 0.05 to 4.36, $p = 0.04$). However, meta-regression analysis (Figure 8; Supplementary Table S1) failed to discover any significant effects on HDL when considering doses, duration, baseline 25OHD and BMI, which was evidenced by the results of permutation tests (Supplementary Table S2).

3.5 Effect of vitamin D supplementation on serum TC level

A total of 1,468 participants from 19 studies (with 22 effect sizes) provided data in terms of serum TC levels. Results did not demonstrate any significant change in TC (−1.56 mg/dL, 95% CI: −7.45 to 4.33, $p = 0.60$; $I^2 = 84.5\%$, $p < 0.01$, Figure 9). For subgroup analysis (Figure 10), significant reductions of TC were observed in participants who were administrated vitamin D supplementation for more than 5,000 IU/day (−10.26 mg/dL, 95% CI: −12.41 to −8.11, $p < 0.01$) and received interventions for less than 24 weeks (−8.14 mg/dL, 95% CI: −12.45 to −3.82, $p < 0.01$). Additionally, vitamin D supplementation significantly decreased TC among participants with a baseline 25OHD level over 50 nmol/L (−7.66 mg/dL, 95% CI: −12.40 to −2.91, $p < 0.01$). However, meta-regressions (Figure 11; Supplementary Table S1) did not reveal potential associations in terms of doses ($p = 0.33$) or duration ($p = 0.08$), but indicated a potential linear regression between BMI and TC (estimate: -2.0005, 95% CI: −3.9893 to −0.0116, $p = 0.05$). Permutation tests did not provide any associations between dose, duration, baseline 25 OHD and BMI with TC levels (Supplementary Table S2).

3.6 Effect of vitamin D supplementation on serum TG level

The meta-analysis assessed 18 studies (21 effects size) with 1,608 participants that provided data on TG. Results highlighted a significant reduction in TG (−8.56 mg/dL, 95% CI: −15.23 to −1.89, $p = 0.01$; $I^2 = 62.0\%$, $p < 0.01$, Figure 12). For subgroup analysis (Figure 13), a significant decrease of TG was observed in participants with BMI lower than 30 (−12.22 mg/dL, 95% CI: −18.86 to −5.59, $p < 0.01$). Moreover, doses over 5,000 IU/day (−9.11 mg/dL, 95% CI: −12.78 to −5.44, $p < 0.01$) and trial duration less than 24 weeks (−10.24 mg/dL, 95% CI: −18.23 to −2.25, $p = 0.01$) significant reduced TG. Studies that did not specify the type of vitamin D supplementation (−9.71 mg/dL, 95% CI: −13.42 to −5.99, $p < 0.01$) and published after 2017 demonstrated a significant decrease in TG (−8.74 mg/dL, 95% CI: −12.78 to −4.70,

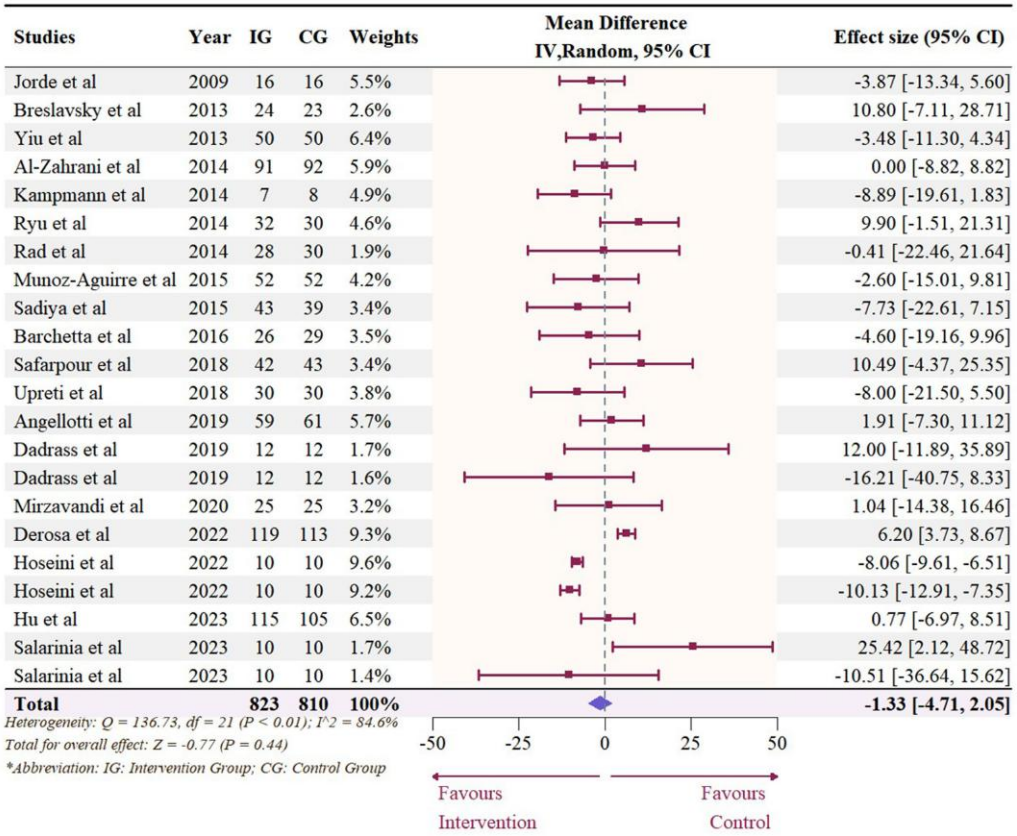

FIGURE 3
Forest plot of the effect of vitamin D supplementation on serum LDL levels among people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval.

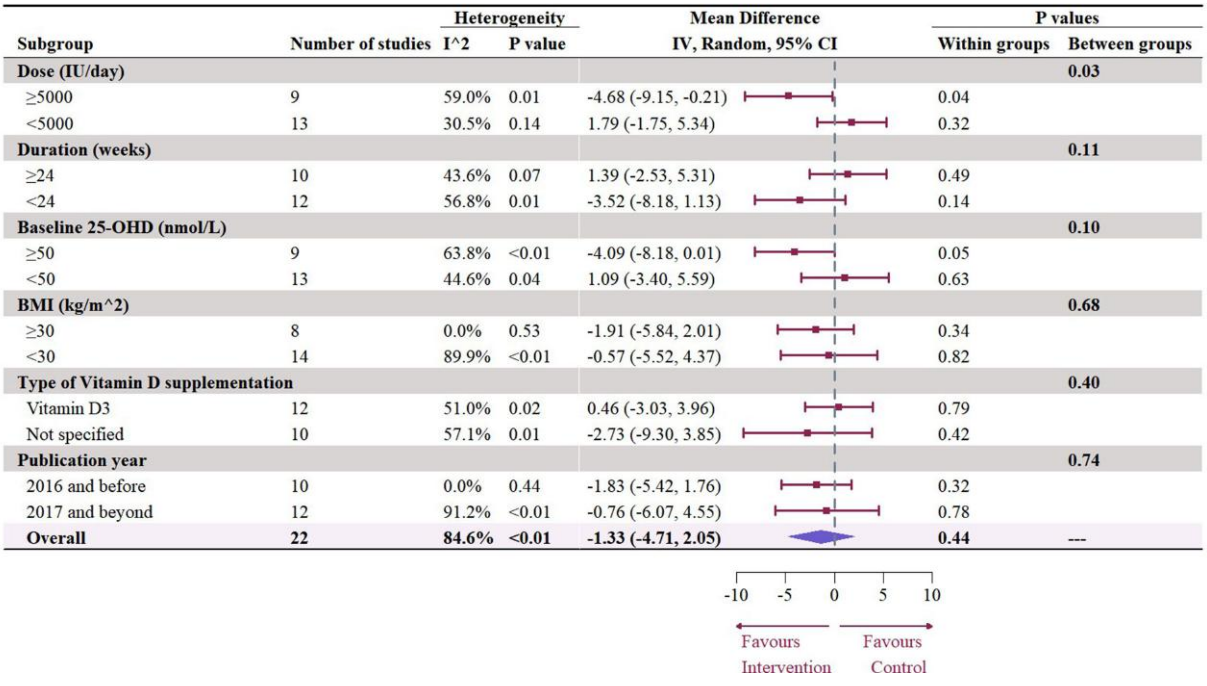

FIGURE 4
Subgroup analysis of the effect of vitamin D supplementation on serum LDL level in people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval; 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

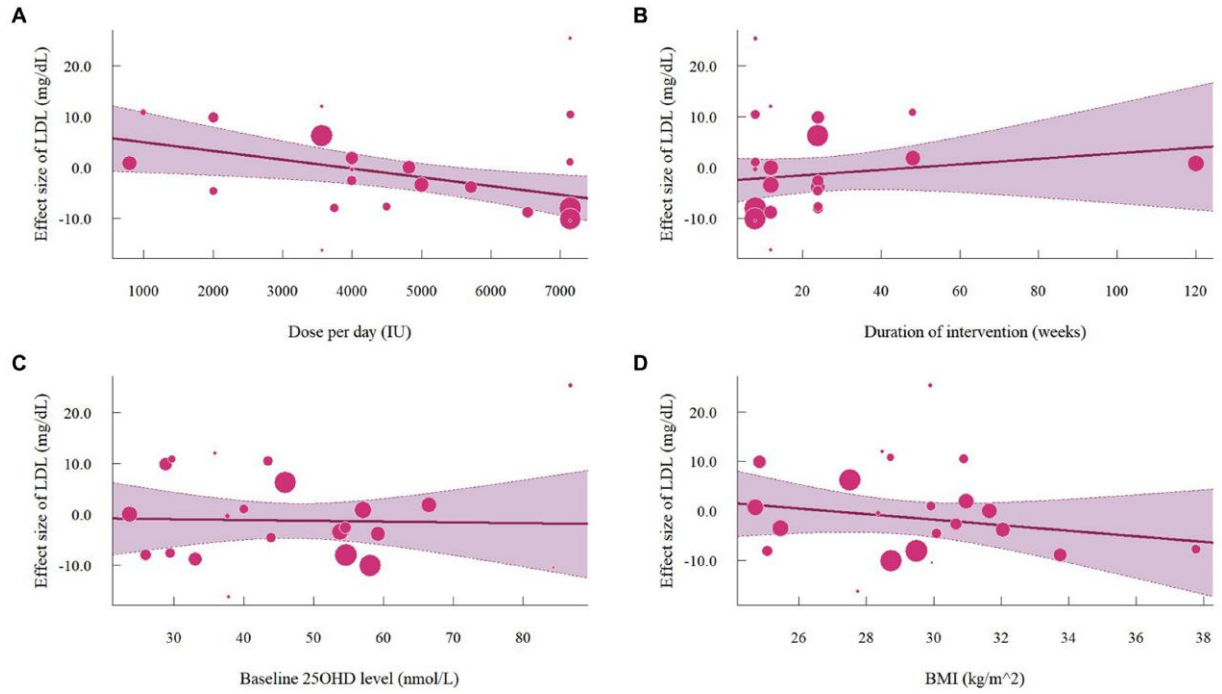

FIGURE 5
Meta-regressions plots of the effect of vitamin D supplementation on LDL and factors: **(A)** Doses; **(B)** Duration; **(C)** 25 OHD; **(D)** BMI. 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

$p < 0.01$). Meta-regression analysis (Figure 14; Supplementary Table S1) indicated a linear association between BMI and TG (estimate: 3.0285, 95% CI: 0.2065 to 5.8506, $p = 0.04$) but permutation test did not reveal any significant associations (Supplementary Table S2).

3.7 Sensitivity analysis

The leave-one-out analyses were conducted and did not find a significant impact on the pooled effect sizes of LDL (Supplementary Figure S1) and TC levels (Supplementary Figure S2) when omitting any single study. The omission of the study conducted by Upreti et al. (38) reduced the overall effect size of TC, but the result was still insignificant. Regarding HDL, the omission of studies conducted by Upreti et al. (38) and two effect sizes from Hoseini et al. (42) resulted in an insignificant overall outcome on HDL (Supplementary Figure S3). In terms of TG (Supplementary Figure S4), leave-one-out analysis revealed that the effects of vitamin D supplementation on TG levels were insignificant after excluding one intervention from Hoseini et al. (42). Given that the changes of effect sizes were not extremely different when omitting one study at a time, the overall results of all meta-analyses were still robust.

3.8 Publication bias

Results of publication bias are listed in Supplementary Table S3, and funnel plots are shown in Supplementary Figure S5. No detectable publication bias was found in LDL (Egger's test: $p = 0.183$; Begg's test: $p = 0.756$) and TG (Egger's test: $p = 0.742$; Begg's test: $p = 0.507$). The funnel plots for LDL and TG showed no apparent asymmetry. However, there was evidence of publication bias in HDL (Egger's test: $p = 0.001$; Begg's test: $p = 0.030$) and TC (Egger's test: $p = 0.026$; Begg's test: $p = 0.272$), which were also indicated by the presence of asymmetry in their respective funnel plots. Trim-and-fill methods were used to predict the potential missing studies for HDL and TC (Supplementary Table S4). In terms of HDL, when using random effect trim-and-fill methods, 6 studies were statistically added and the overall effect size was still significant (2.11 mg/dL, 95% CI: 0.80 to 3.42, $p < 0.01$). Fixed-effect trim-and-fill method statistically added 11 missing studies and the final effect size was still significant (4.67 mg/dL, 95% CI: 2.72 to 6.63, $p < 0.01$). For TC, although Egger's test suggested the presence of publication bias in studies on TC, the trim-and-fill method was unable to account for potential missing studies when employing a random effects model. When applying the fix-effect trim-and-fill method, 9 missing studies were statistically added and the final effect size became significant (−10.76 mg/dL, 95% CI: −17.80 to −3.71, $p < 0.01$).

4 Discussions

In this meta-analysis, we included 20 RCTs investigating the effects of vitamin D supplementation on lipid profiles in people with type 2 diabetes. The findings showed a significant increase in HDL and a decrease in TG levels. However, no significant impact was observed on LDL and TC levels. Our results are similar to the findings from previous meta-analyses. One meta-analysis on the general population demonstrated significant improvement of vitamin D in TC, LDL, and TG levels, while not in HDL levels (45). For the population with pre-diabetes, a meta-analysis revealed a significant decrease in TG

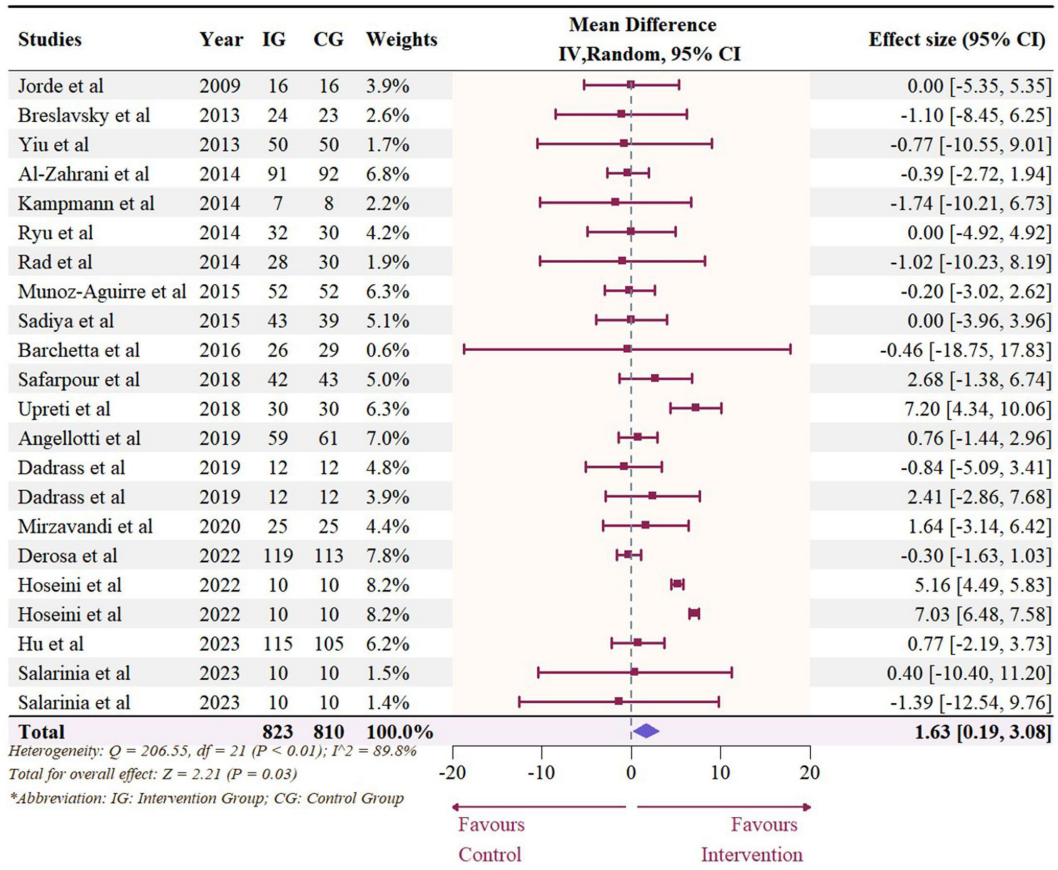

FIGURE 6
Forest plot of the effect of vitamin D supplementation on serum HDL levels among people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval.

levels, but no significant change in LDL, HDL, and TC levels (46). Nevertheless, a meta-analysis conducted on adults with metabolic syndrome failed to find any significant alteration in LDL, HDL, TC, and TG after the implementation of vitamin D supplementation (47). Therefore, the effects of vitamin D on lipid profiles may vary depending on the specific conditions that participants are afflicted with.

The previous meta-analysis of the same topic by Jafari et al. (6) in 2016 reported improvements in TG and TC levels but not LDL and HDL levels. The differences may be explained by the various application of vitamin D. It is worth noting, that the previous meta-analysis included trials employing both fortification and supplementation. In their meta-analysis, all studies that applied vitamin D fortification were less than 2000 IU/day but demonstrated enhanced effects on lipid profiles (6). The absorption of vitamin D is optimized when consumed in conjunction with foods rich in dietary fat (48), and vitamin D fortification is often taken with fat-containing foods (49, 50), which may explain why vitamin D fortification may yield different results compared to vitamin D supplementation. Additionally, we examined the differences among studies published before and after 2017. For studies before 2017, our findings align with the subgroup analysis results reported by Jafari et al. (6). However, studies after 2017 indicated a potential to improve HDL and TG but not LDL and TC. Intriguingly, our findings from subgroup analyses by type also reported similar outcomes. Among the studies that did not specify the type of vitamin D supplementation, five studies were published after 2017. In this sense, the observed enhancement in studies after 2017 could potentially be linked to the unspecified type. It may, however, raise concerns about the potential overestimation of true effects due to the lack of reporting on the specific type.

Subgroup analyses under doses revealed a significant improvement across lipid profiles with the administration of higher doses. Both meta-regressions and permutation tests confirmed the positive effect of doses in improving LDL levels, thereby reinforcing the robustness of its association. Considering that the current aim of treating and preventing dyslipidemia is to improve serum LDL levels (51), our meta-analysis highlights the potential of higher doses of vitamin D supplementation in reducing dyslipidemia. Additionally, potential improvements in lipid profiles are more likely to occur in shorter trial duration (< 24 weeks). Similar results were demonstrated in many previous meta-analyses, which may be attributed to reduced adherence to vitamin D administration in long-term trials (6, 45, 52, 53). Moreover, considering the half-life of vitamin D is approximately 2 months, its therapeutic effects are typically achieved and maintained within a short period (53). Nevertheless, only 3 studies implemented interventions for 48 weeks (12, 29, 40). Consequently, there is still a dearth of evidence regarding the impact of longer durations.

Moreover, participants with a baseline BMI < 30 kg/m^2 are more likely to derive lipid profile improvements from vitamin D supplementation, especially on TG levels. Due to the fat-soluble nature of vitamin D, it is more likely to be sequestrated in adipose tissue (54). Thus, individuals with a lowerBMI may exhibit a higher concentration of 25 OHD serum level when administered the same dosage of vitamin D supplementation, compared to people with a higher BMI. However,

Subgroup	Number of studies	Heterogeneity I^2	Heterogeneity P value	Mean Difference IV, Random, 95% CI	P values Within groups	P values Between groups
Dose (IU/day)						**0.11**
≥5000	9	78.8%	<0.01	3.11 (0.69, 5.53)	0.01	
<5000	13	51.2%	0.02	0.80 (-0.67, 2.27)	0.29	
Duration (weeks)						**0.40**
≥24	10	60.7%	0.01	1.00 (-0.79, 2.78)	0.28	
<24	12	85.4%	<0.01	2.20 (0.05, 4.36)	0.04	
Baseline 25-OHD (nmol/L)						**0.42**
≥50	9	90.0%	<0.01	2.27 (-0.11, 4.65)	0.06	
<50	13	53.3%	0.01	1.06 (-0.67, 2.79)	0.23	
BMI (kg/m^2)						**0.09**
≥30	8	0.0%	0.95	0.29 (-0.92, 1.49)	0.64	
<30	14	90.8%	<0.01	2.30 (0.31, 4.28)	0.02	
Type of Vitamin D supplementation						**<0.01**
Vitamin D3	12	0.0%	1.00	-0.05 (-0.90, 0.80)	0.90	
Not specified	10	78.8%	<0.01	3.69 (1.55, 5.82)	<0.01	
Publication year						**0.01**
2016 and before	10	0.0%	1.00	-0.32 (-1.73, 1.08)	0.65	
2017 and beyond	12	92.6%	<0.01	2.71 (0.80, 4.62)	0.01	
Overall	**22**	**89.8%**	**<0.01**	**1.63 (0.19, 3.08)**	**0.03**	---

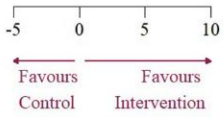

FIGURE 7
Subgroup analysis of the effect of vitamin D supplementation on serum HDL level in people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval; 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

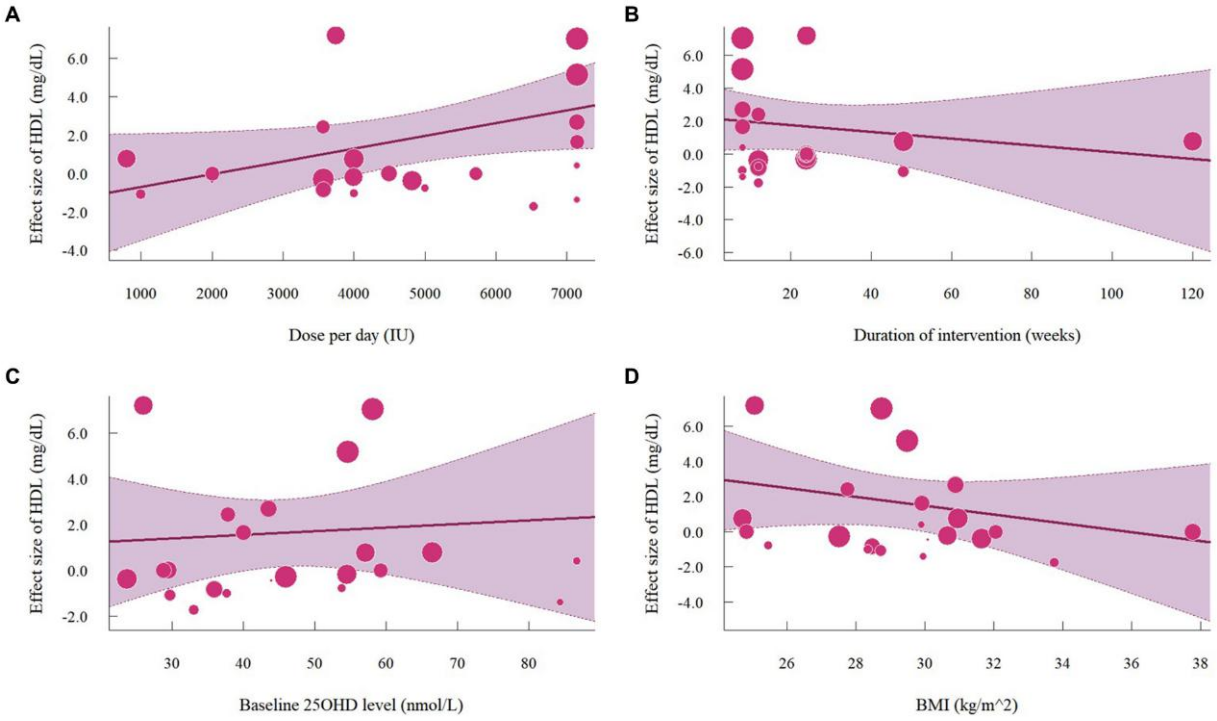

FIGURE 8
Meta-regressions plots of the effect of vitamin D supplementation on HDL and factors: **(A)** Doses; **(B)** Duration; **(C)** 25 OHD; **(D)** BMI. 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

permutation tests indicated that the potential associations between BMI and TC or TG may be susceptible to false positive findings. The permutation test is aimed to control the type 1 error rate, and thus often results in higher *p* values compared to meta-regressions (24). Also, participants with 25 OHD levels of more than 50 nmol/L experienced significant improvements in TC and TG levels. Jafari et al. (6) previously discovered that vitamin D may be more effective in improving TC and LDL among people with T2D and insufficient or sufficient serum

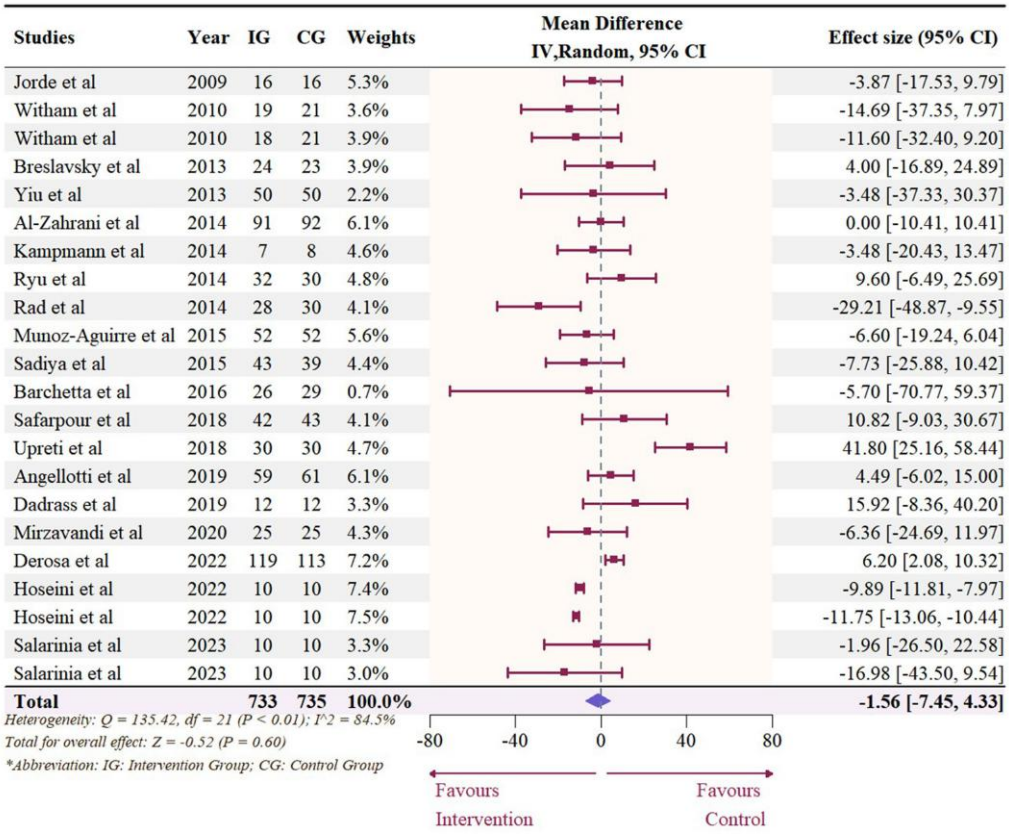

FIGURE 9
Forest plot of the effect of vitamin D supplementation on serum TC levels among people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval.

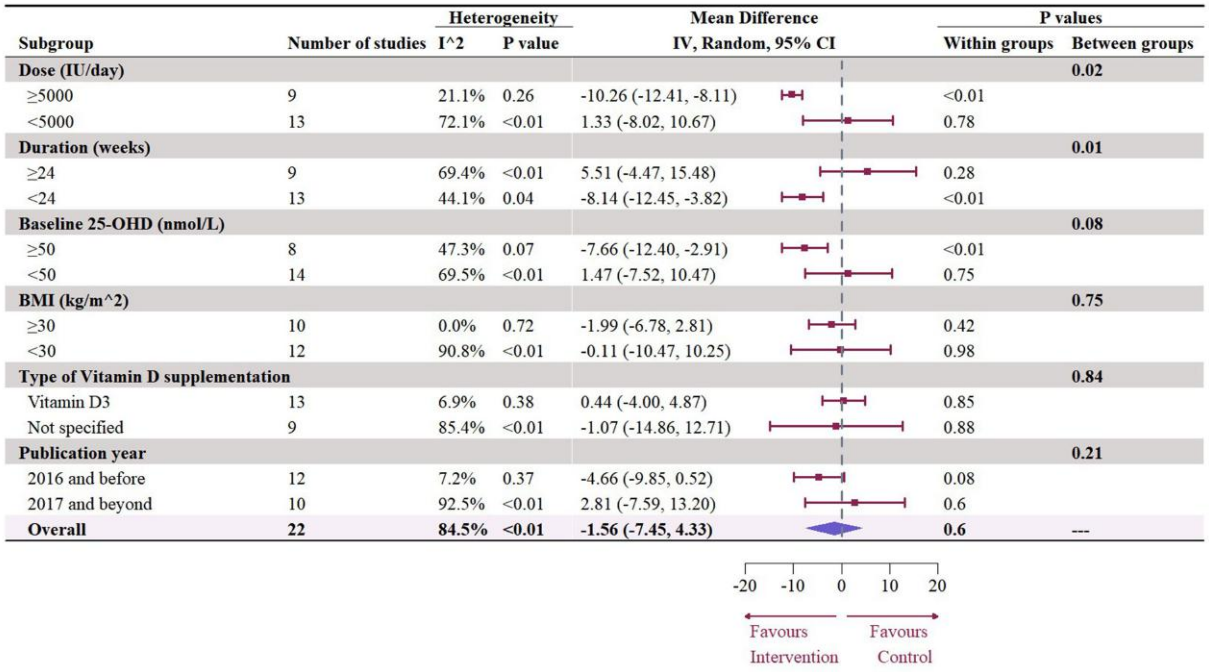

FIGURE 10
Subgroup analysis of the effect of vitamin D supplementation on serum TC level in people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval; 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

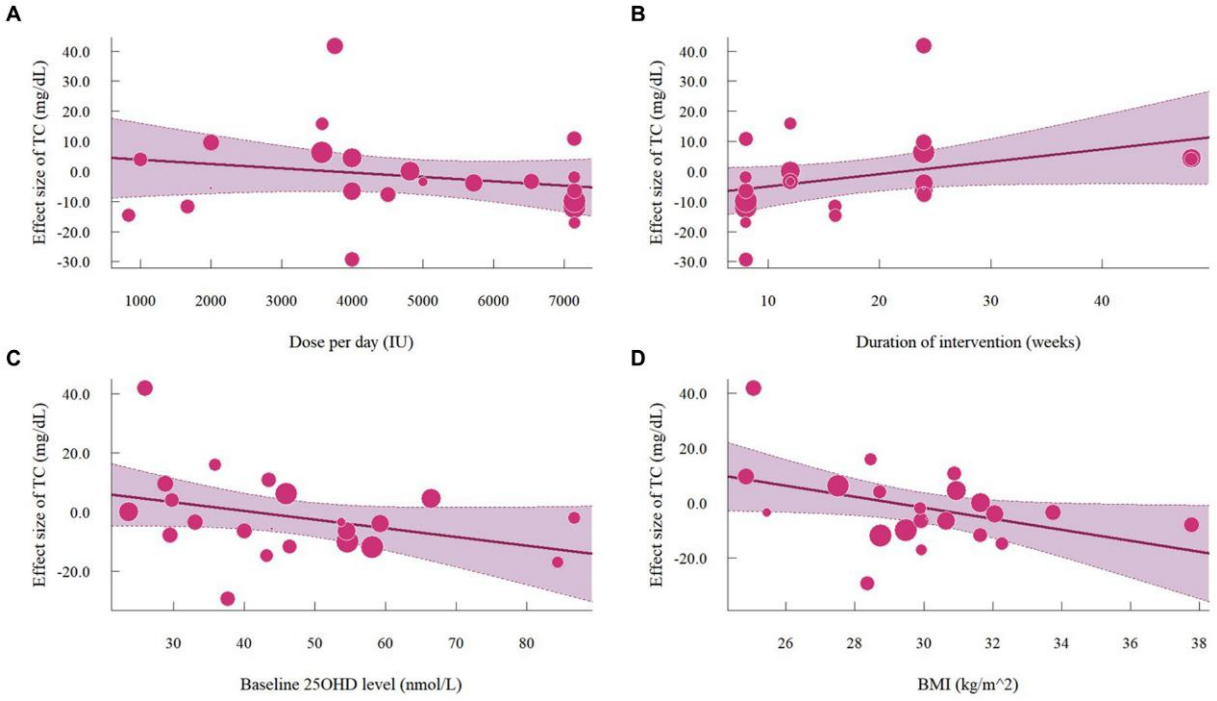

FIGURE 11
Meta-regressions plots of the effect of vitamin D supplementation on TC and factors: **(A)** Doses; **(B)** Duration; **(C)** 25 OHD; **(D)** BMI. 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

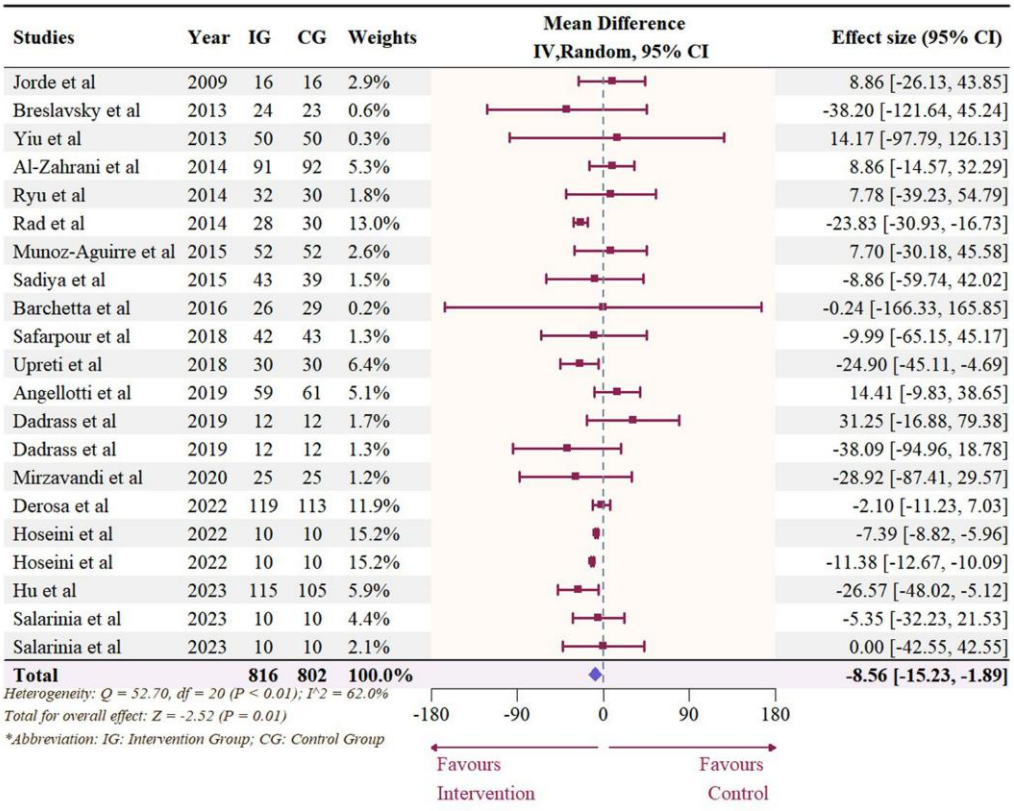

FIGURE 12
Forest plot of the effect of vitamin D supplementation on serum TG levels among people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval.

Subgroup	Number of studies	Heterogeneity I^2	Heterogeneity P value	Mean Difference IV, Random, 95% CI	P values Within groups	P values Between groups
Dose (IU/day)						**0.85**
≥5000	8	62.1%	0.01	-9.11 (-12.78, -5.44)	<0.01	
<5000	13	62.2%	<0.01	-7.97 (-19.26, 3.33)	0.17	
Duration (weeks)						**0.58**
≥24	10	27.4%	0.19	-6.17 (-18.23, 5.90)	0.32	
<24	11	74.2%	<0.01	-10.24 (-18.23, -2.25)	0.01	
Baseline 25-OHD (nmol/L)						**0.78**
≥50	9	68.0%	<0.01	-8.72 (-12.74, -4.70)	<0.01	
<50	12	54.5%	0.01	-10.50 (-22.16, 1.16)	0.08	
BMI (kg/m^2)						**0.01**
≥30	7	0.0%	0.98	8.04 (-5.10, 21.19)	0.23	
<30	14	70.8%	<0.01	-12.22 (-18.86, -5.59)	<0.01	
Type of Vitamin D supplementation						**0.06**
Vitamin D3	11	0.0%	0.51	-1.01 (-11.36, 9.34)	0.85	
Not specified	10	76.5%	<0.01	-13.28 (-20.43, -6.14)	<0.01	
Publication year						**0.65**
2016 and before	9	0.0%	1.00	-4.95 (-20.94, 11.04)	0.54	
2017 and beyond	12	40.8%	0.10	-8.74 (-12.78, -4.70)	<0.01	
Overall	**21**	**62.0%**	**<0.01**	**-8.56 (-15.23, -1.89)**	**0.01**	---

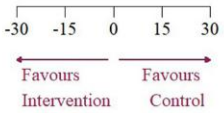

FIGURE 13
Subgroup analysis of the effect of vitamin D supplementation on serum TG level in people with type 2 diabetes. IV, inverse variance weighted; CI, confidence interval; 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

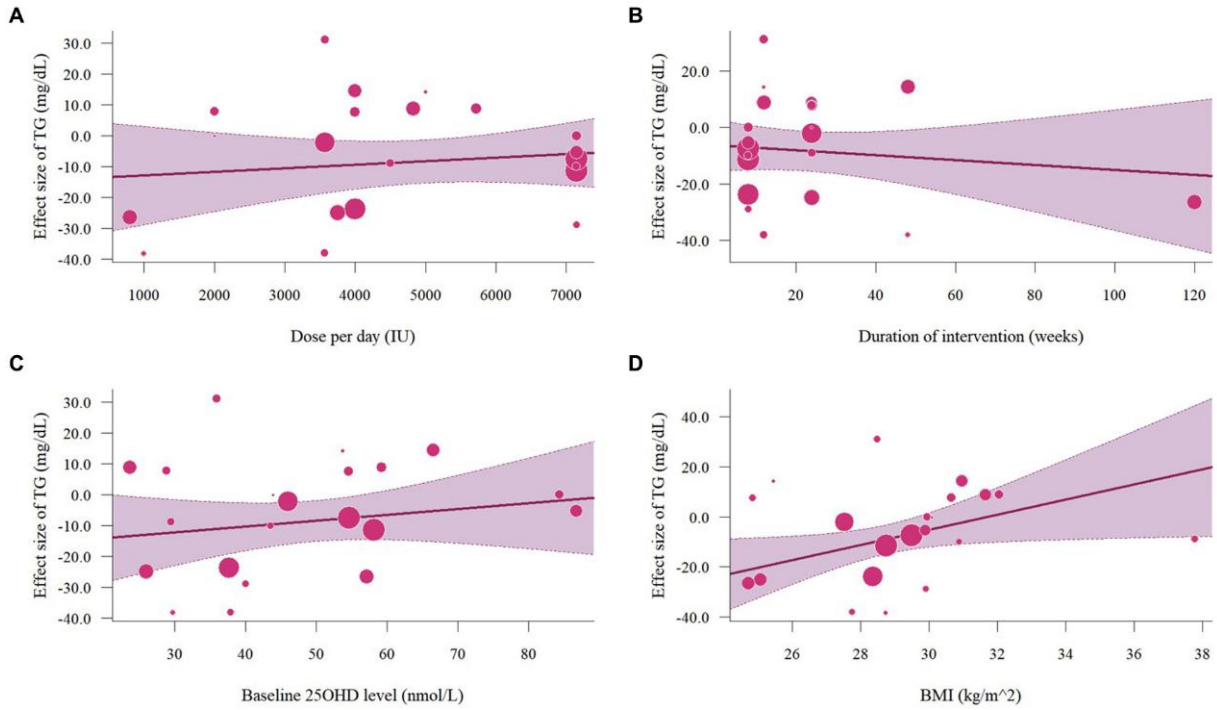

FIGURE 14
Meta-regressions plots of the effect of vitamin D supplementation on TG and factors: **(A)** Doses; **(B)** Duration; **(C)** 25 OHD; **(D)** BMI. 25 OHD, 25-hydroxy vitamin D; BMI, body mass index; IU, international unit.

vitamin D status. This may be because only four studies regarding 25 OHD ≥ 50 nmol/L were included in their meta-analysis (6).

In this current study, inconsistent results in subgroup analysis and meta-regression analysis were only observed regarding TC, which may be due to the impact of outliers (55). Sensitivity analyses revealed that the study by Upreti et al. (38) and two effect sizes from Hoseini et al. (42) were more likely to be considered as potential outliers. In the study of Upreti et al. (38), 60 participants (30 in each arm) were recruited in a

24-week intervention. The TC levels between the intervention group and the control group at baseline were considerably different (no statistical analysis was performed), but were similar after the intervention. As such, this significant increase in TC was more likely attributed to the difference in baseline TC levels rather than the effects of vitamin D. Hoseini et al. (42) conducted an 8-week single-blinded RCT using aerobic training (AT) and vitamin D supplementation comprised four groups (AT+ vitamin D; AT; Vitamin D; placebo). The baseline lipid profiles were comparable among the four groups; however, the significant effects of vitamin D supplementation on lipid profiles may vary due to the limited number of participants included (10 in each arm).

Publication biases were observed in HDL and TC, thereby the trim-and-fill method was performed. All hypothetical compensated studies demonstrated favorable impacts on HDL and TC levels. Most studies analyzed for TC overlapped with those in the analysis of TG and LDL, and the studies examining the effects on HDL were identical to those used for LDL assessment. Given that no publication bias was detected in terms of LDL and TG, it seemed unlikely that the absences of compensated studies in HDL and TC were caused by publication bias. Rather, the asymmetry discovered in funnel plots was more likely caused by other factors such as high heterogeneity between studies or different quality of studies (56). Notably, since the heterogeneity of meta-analyses regarding HDL and TC was relatively high, the results of the trim-and-fill method may not be accurate (56).

There are some plausible mechanisms through which vitamin D could potentially modulate HDL and TG. Firstly, vitamin D could potentially inhibit the expression of nuclear factor sterol regulatory element-binding protein 1c, which plays a role in hepatic triglyceride synthesis (46). Additionally, vitamin D may upregulate lipoprotein lipase (LPL) in muscle and fat tissues. A cross-sectional study has shown a positive association between serum vitamin D levels with LPL (57), and activation of LPL would further increase clearance of circulating lipoprotein particles (6, 46). Third, increasing vitamin D serum levels may improve TG and HDL in people with T2D. Vitamin D deficiency potentially impacts the functioning of beta cells and insulin resistance, consequently affecting lipoprotein metabolism and leading to elevated TG levels and decreased HDL levels (58). Lastly, vitamin D may reduce TG levels by regulating parathyroid hormone (PTH). The elevation of PTH levels may lead to an increase in the production of TG, and the presence of vitamin D inhibits the secretion of PTH in the bloodstream (58, 59).

A strength of this meta-analysis is that most included studies sustained a moderate to high quality, with low drop-out rates and followed per-protocol analyses. Thus, our study highlighted the value of vitamin D supplementation in the management of CVD, particularly in its role in regulating dyslipidemia, among people with T2D. Moreover, permutation tests were applied to enhance the robustness of our results. However, there are some limitations which should be further considered. Firstly, the heterogeneity between studies was high. It may be due to dissimilar baseline lipid profiles among participants, different study designs of interventions, and various ethnicities of included populations. Second, due to the lack of baseline BMI or 25 OHD data, not all potentially eligible studies were included in this meta-analysis, which may affect the generalizability of the outcome.

5 Conclusion

In conclusion, this meta-analysis demonstrated that vitamin D supplementation can significantly increase HDL levels and decrease TG levels among people with type 2 diabetes. However, vitamin D supplementation failed to improve LDL and TC levels. The potential benefits of lipid profiles from vitamin D supplementation are more likely to be observed with shorter intervention periods or higher doses of vitamin D administration. While some potential effects of vitamin D supplementation have been noted in this study, additional long-term and rigorous RCTs are required to validate clinical significance. Moreover, further studies should also consider the influence of factors such as doses, type of vitamin D supplementation, participants' BMI and serum 25OHD levels on the effects of vitamin D on lipid profiles among people with T2D.

Data availability statement

The original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1419747/full#supplementary-material

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Vitamin D status, vitamin D receptor, CYP2R1, and CYP24A1 profiles in children

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Introduction: Vitamin D plays a major role in the musculoskeletal and immune system. Understanding the comprehensive mechanism of vitamin D receptors and the enzyme of vitamin D induction (CYP2R1) and inhibition (CYP24A1) in its metabolism is interesting. This study aims to understand vitamin D metabolism in Indonesian pediatrics, specifically in Jakarta, which has abundant sun exposure.

Methodology: A cross-sectional study with comparative, correlative, and multivariate analysis on vitamin D, vitamin D receptor, CYP2R1, and CYP24A1 levels was conducted on 46 children with no known morbidity.

Result: Subjects were mostly male (52.2%), age group of 2–6 years (34.8%), and had sufficient vitamin D status (43.5%, median 27.55 ng/mL). Age was found to have a negative correlation with vitamin D levels ($p < 0.001$; $r = -0.625$) and CYP2R1 ($p = 0.035$; $r = -0.311$). Significant positive associations were found between CYP24A1 and CYP2R1 ($p = 0.046$; $r = 0.296$). Participants aged 0–2 are more likely to have a higher level of vitamin D status compared to those aged >2 years (OR 42.092, 95% CI [4.532–390.914], $p = 0.001$). VDR levels were significantly lower in insufficient vitamin D levels than in the sufficient group ($p = 0.018$). VDR and vitamin D status had a positive relation (OR 7.023, 95% CI [1.864–26.453], $p = 0.004$).

Conclusion: Vitamin D levels decrease with the increase in age. Vitamin D receptor level has an inline-level progression with vitamin D level. CYP2R1 and CYP24A1 suggest a directly proportional relationship. Vitamin D screening and supplementation in children older than 2 years old are suggested.

KEYWORDS

vitamin D, vitamin D receptor, CYP2R1, CYP24A1, pediatric, adolescence

Introduction

Vitamin D is categorized into vitamin D deficiency (<20 ng/mL), insufficiency (21–30 ng/mL), and sufficiency (>30 ng/mL). The prevalence of vitamin D has been widely studied among continents (1, 2). Vitamin D deficiency is not limited to non-tropical, but also in tropical countries, which have abundant sunlight (3, 4). Vitamin D is a principal factor in bone and

neuron health, immunity, cancer, and cardiovascular disease (5). Vitamin D deficiency in children increases the risk of rachitis, seizure, hypocalcemia, and delayed tooth growth. In adolescence, vitamin D deficiency is associated with musculoskeletal pain, weak muscle tone, lower extremity deformity, and increased risk of acute or chronic diseases (6). Furthermore, vitamin D deficiency is related to obesity, cardiovascular risk, insulin resistance, beta cell dysfunction, autoimmune diseases, and cancer. Factors contributing to vitamin D levels include sunlight exposure, skin pigmentation, diet, and vitamin D supplementation (7, 8).

In Southeast Asia, 1 in 2 neonates and more than 60% of adolescents have vitamin D deficiency. In Indonesia and Thailand, 9 of 10 neonates have vitamin D deficiency (6). The percentage of Indonesian children with insufficient and inadequate vitamin D is relatively higher compared to Malaysia, Thailand, and Vietnam. Research on vitamin D deficiency in Indonesia has not been done widely. However, research in 2015 of children aged 2–12 years old in 47 Indonesian districts showed that 44% was insufficient, 50.3% was inadequate, and only 5.6% was sufficient in vitamin D (9). Abboud et al. stated that factors affecting vitamin D 25(OH)D level in circulation, such as cholesterol synthesis, hydroxylation, and vitamin D transport, is mediated by cytochrome P450 or CYP450, including CYP2R1 and CYP24A1 (10). The metabolism of vitamin D seems to be affected by race or ethnicity. Prior studies indicated that racial and ethnic differences are evident in the markers of vitamin D metabolism, which can be attributed, at least in part, to genetic heritage (11). In addition, other factors including gene polymorphism and locus variation, vitamin D hydroxylation epigenetic regulation (Deoxyribonucleic acid or DNA methylation), calcium intake, and the density of fat and muscle tissue also contribute to vitamin D levels (12, 13).

Provitamin D3 in the skin (7-dehydrocholesterol) and D2 (ergosterol) from the diet are the main sources of vitamin D. Ultraviolet B (UVB) from sunlight is required to convert 7-dehydrocholesterol into cholecalciferol (vitamin D3). Ergosterol will be converted into ergocalciferol (vitamin D2) (14). The enzymatic conversion in the liver is mediated by CYP2R1, which helps the formation of calcidiol (25(OH)D) (15). Following interaction with 1α hydroxylase in the renal, calcidiol becomes its active form, 1,25(OH)2D3 (calcitriol). Calcitriol binds to vitamin D receptors (VDR) to function in each organ (2). Once vitamin D concentration is high, the synthesis is reduced by inducing the CYP24A1 activity which inactivates and accelerates the catabolism of calcitriol (16).

Although the importance of vitamin D in children and the influence of demographic background on vitamin D levels has been widely acknowledged, there has been no study regarding vitamin D, VDR, CYP2R1, and CYP24A1 in Indonesian pediatrics. This study aims to analyze the relation of vitamin D levels with VDR and CYP450 (CYP2R1 and CYP24A1) levels in Indonesian children.

Materials and methods

Study design

This research is a cross-sectional study conducted in Bunda Woman and Children Hospital, Jakarta, Indonesia. The subjects included in this study were 46 subjects. Inclusion criteria were < 18 years old; without comorbidities and chronic disease, which was determined based on physical examination and complete blood test. Those who declined research participation were excluded. Obtained data included gender, age, vitamin D, VDR, CYP2R1, and CYP24A1. The measured variables, vitamin D, VDR, CYP2R1, and CYP24A1, were analyzed based on age group: 0–2 years old, 2–6 years old, and 6–18 years old.

The sample size was calculated using the formula of correlative analytic research and MedCalc application (17). This research used a 95% confidence interval (CI), β of 10%, and a correlation coefficient of 0.5. Therefore, the applied Zα and Zβ were 1.96 and 1.282, respectively (18).

$$n = \left[\frac{(Z\alpha + Z\beta)}{0.5\ln\left(\frac{1+r}{1-r}\right)}\right]^2 + 3 = \left[\frac{(1.96+1.282)}{0.5\ln\left(\frac{1+0.5}{1-0.5}\right)}\right]^2 + 3 = 37.84 \approx 38$$

Measurement

Vitamin D level measurement was completed by examining 25 (OH) D serum levels. A total of 3–5 mL of the sample's blood was placed in a serum tube from a cuffed venous sample. The tool used for this examination was Roche Diagnostics' Cobas e411 which was a combination of competitive immunoassay and enzyme-catalyzed chemiluminescence detection (CLIA test). Examination of VDR and CYP24A1 serum levels was carried out using the ELISA method and reagen from Elabscience, USA (Catalog No: E-EL-H2043 and E-EL-H0377). ELISA method was also used for CYP2R1 serum levels examination reagen from Abebio, China (Catalog No: AE48038HU). All procedures were carried out based on the manufacturer's recommendation.

Statistical analysis

Data were analyzed using Statistical Product and Service Solutions (SPSS) application system version 26.0. The data normality test was carried out on all subjects using Shapiro–Wilk (n < 50). Data were normally distributed if the *p*-value >0.05. Mean and standard deviation were used in normally distributed data; otherwise, median and interquartile range (IQR) were applied. Bivariate comparative analysis was carried out using an independent T-test (≤2 groups) or One Way Anova (>2 groups) in normally distributed data. Meanwhile, the Mann–Whitney test (≤2 groups) or the Kruskal-Wallis test (>2 groups) was used in abnormal data distribution. The correlation analysis between subject characteristics (gender, age, and vitamin D status), vitamin D levels, VDR, CYP2R1, and CYP24A1 levels was carried out. Correlation tests were completed using Pearson, Spearman, or Eta correlation, depending on the variable type. *p*-values <0.05 were considered statistically significant. Significant findings on correlation tests were further analyzed using *post hoc* analysis. Multivariate analysis of vitamin D status was conducted using ordinal regression analysis and generalized linear model. The factors included

were sex and age group. The covariates included were the levels of VDR, CYP2R1, and CYP24A1.

Ethical approval

The aim of the study had been explained and assent consent had been obtained from all subjects. This research was approved based on the Helsinki Declaration by the Ethics Commission of the Faculty of Medicine, YARSI University (No: 030/KEP-UY/EA.20/II/2023).

Results

A total of 46 subjects(<18 years old) data, collected from Bunda Woman and Children Hospital, Jakarta, Indonesia, were included in this study. Subjects were mostly male (52.2%), age group of 2–6 (34.8%), and had sufficient vitamin D status (43.5%). Subject demographic based on vitamin D status (Table 1), showed that 50% of males and females had sufficient and insufficient vitamin D status, respectively. Based on age, the age group of 0–2 years mostly had sufficient vitamin D status (86.7%), meanwhile, the 2–18 years had insufficient vitamin D status.

The vitamin D level median among the subjects was 27.55 ng/mL. Significant differences were found in vitamin D levels based on age ($p < 0.001$). The highest vitamin D was found in 0–2 years old, 37.35 [8.27] ng/mL, and the lowest in 6–18 years old, 23.30 (6.30) ng/mL. Mann Whitney *post hoc* test was conducted and resulted in significantly higher vitamin D levels in age group 0–2 years in comparison to 2–6 years ($p = 0.022$) and 6–17 years ($p < 0.001$) (Figure 1A). VDR level was significantly different based on vitamin D status ($p = 0.031$) with the highest mean found in the sufficient group (1.75 [0.787] ng/mL). The analysis was followed with the Games-Howell *post hoc* test and resulted in VDR levels in sufficient vitamin D status being higher in comparison to those in insufficient group (Figure 1B; $p = 0.018$). There was no significant value comparison of CYP2R1 and CYP24A1 based on gender, age, and vitamin D status. Subject characteristics and the comparative test results are presented in Table 2.

Correlation bivariate analysis was conducted based on subject characteristics. Age was found to have a negative correlation with vitamin D levels ($p < 0.001$; $r = -0.625$) and CYP2R1 ($p = 0.035$; $r = -0.311$). The scatterplot of the correlation between age and vitamin D, VDR, CYP2R1, and CYP24A1 is provided in Figure 2. VDR and vitamin D status had a positive correlation ($p = 0.013$; $r = 0.363$). There was no significant association finding on CYP24A1 based on subject characteristics. Between measured variables, which were vitamin D, VDR, CYP2R1, and CYP24A1, a bivariate correlation test was carried out. Significant positive correlations were found between vitamin D level and VDR level ($p = 0.006$; $r = 0.399$) and in CYP24A1 and CYP2R1 ($p = 0.046$; $r = 0.296$). Complete correlation test results are depicted in Table 3.

The ordinal multivariate analysis was conducted to examine the association between vitamin D status, sex, age, and VDR. CYP2R1, and CYP24A1 level. The ordinal regression analysis showed that the included independent variables influence vitamin D status up to 62.0% (pseudo $R^2 = 0.620$). The generalized linear model suggests that VDR (OR 7.023, 95% CI [1.864–26.453], $p = 0.004$) is positively associated with vitamin D level. The estimate from Table 4 suggests that participants aged 0–2 are more likely to have a higher level of vitamin D status compared to participants aged > 2 years (OR 42.092, 95% CI [4.532–390.914], $p = 0.001$). There were no significant associations between the level of CYP2R1 and CYP24A1 and sex. The multivariate analysis results are described in Table 4.

Discussion

This study aims to understand the relationship between vitamin D level, VDR, CYP2R1, and CYP24A1. Vitamin D deficiency is common in South East Asia pediatrics (6). Previous research in an Indonesian pediatric sample aged 7–12 years in Jakarta indicated that most children were insufficient in vitamin D (19). Other research showed that in the age group of 2.0–12.9 years vitamin D mean was inadequate (9). However, the current research indicated that most of the participants had sufficient vitamin D status (43.5%). This discrepancy can be explained by different age groups of subjects. Our subjects had a wider age range (<18 years) and were normally distributed between each age range. Younger age is known to have higher vitamin D levels, which might elevate the whole sample's mean (20). Furthermore, the global consensuses, for example, World Health Organization (WHO), Centers for Disease Control and Prevention (CDC), and Paediatric Association, on vitamin D supplementation as prophylaxis have been applied widely. These guidelines focus on preventing rickets, infections, and other chronic disease (21). The recommended vitamin D supplementation dose in infants is 400 IU. However, vitamin D dose recommendations ideally differ

TABLE 1 Subject demographic based on vitamin D status.

	n (%)	Vitamin D status		
		Deficient (%)	Insufficient (%)	Sufficient (%)
Sex				
Male	24 (52.9)	5 (20.8)	7 (29.2)	12 (50.0)
Female	22 (47.8)	3 (13.6)	11 (50.0)	8 (36.4)
Age (years)				
0 to <2	15 (32.6)	0 (0.0)	2 (13.3)	13 (86.7)
2 to <6	16 (34.8)	3 (18.8)	7 (43.7)	6 (37.5)
6–18	15 (32.6)	5 (33.3)	9 (60.0)	1 (6.7)

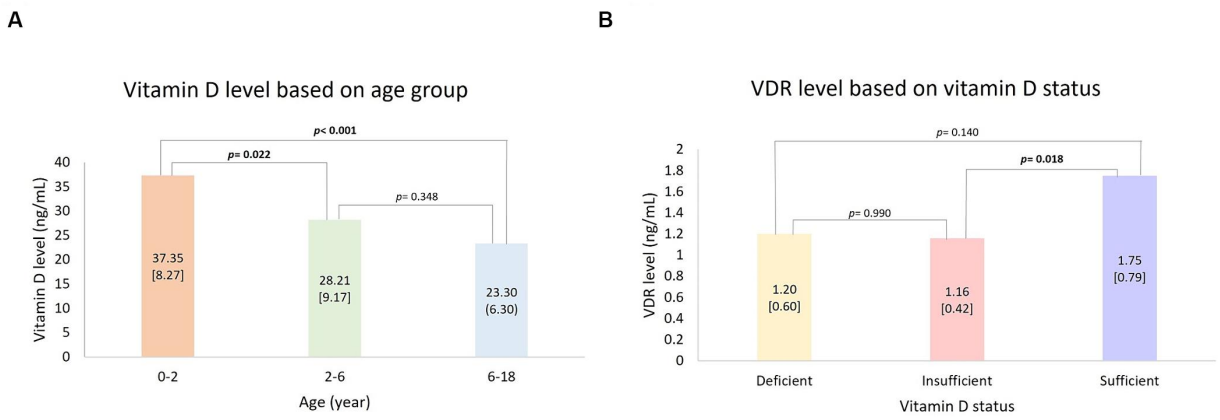

FIGURE 1
Post hoc analysis: **(A)** Vitamin D level based on age group and **(B)** VDR level based on vitamin D status. Bold value indicates a statistically significant comparison.

TABLE 2 Subject characteristics with vitamin D, VDR, CYP2R1, and CYP24A1 levels.

Variables	*n* = 46 (%)	Vitamin D (ng/mL)	*p*-value	VDR (ng/mL)	*p*-value	CYP2R1 (ng/mL)	*p*-value	CYP24A1 (ng/mL)	*p*-value
Sex									
Male	24 (52.2)	30.26 [8.97]	0.645	1.50 [0.67]	0.441	20.95 (28.91)	0.947	0.48 [0.25]	0.787
Female	22 (47.8)	28.93 [10.45]		1.13 (0.55)		19.89 (27.01)		0.50 [0.21]	
Age (years)									
0 to <2	15 (32.6)	37.35 [8.27]	<0.001*	1.46 [0.69]	0.286	22.86 (78.02)	0.110	0.40 (0.24)	0.207
2 to <6	16 (34.8)	28.21 [9.17]		1.60 [0.76]		24.20 [14.19]		0.56 [0.17]	
6–18	15 (32.6)	23.30 (6.30)		1.21 [0.57]		15.91 (20.15)		0.45 [0.29]	
Vitamin D status									
Deficient	8 (17.4)	18.25 [1.88]	<0.001*	1.20 [0.60]	0.031*	20.37 [8.19]	0.122	0.54 [0.25]	0.710
Insufficient	18 (39.1)	24.67 [2.75]		1.16 [0.42]		15.66 (28.73)		0.51 [0.23]	
Sufficient	20 (43.5)	37.70 (8.88)		1.75 [0.79]		22.27 (34.63)		0.46 [0.24]	

Mean [SD] or Median (IQR); IQR, interquartile range; SD, standard deviation; VDR, vitamin D receptor; CYP, cytochrome.
p-value denotes the comparison analysis between variables (sex, age, and vitamin D status) and the level of vitamin D, VDR, CYP2R1, and CYP24A1. *$p < 0.05$ denotes statistical significance.

between age groups and predispose factors, ranging from 400–4,000 IU/day or 10–100 μg/day (22).

Vitamin D levels were significantly higher in the age group 0–2 years in comparison to the age group of 2–6 years and 6–18 years. The age group of 0–2 years was found to be one of the vitamin D predictors. Age 0–2 years were found to have the highest vitamin D level. Vitamin D deficiency was prevalent in children aged 6–12 years in Busan with a mean of 14.86 ± 3.20 ng/mL (23). Other studies showed that in comparison to preschoolers, a considerably higher percentage of elementary school students and teenagers fell into the category of vitamin D deficiency and overall low vitamin D (20). In Indonesia, a previous study showed that younger children had better vitamin D mean. Ernawati et al. found that vitamin D in the age group of 2.0–2.9 (54 ± 2.3 nmoL/L) was higher than in the age group of 9.0–12.9 (50.3 ± 1.4 nmoL/L) (9). The age group of 6–18 years was found to have the lowest vitamin D level in this study. It can be partially explained by the increasing vitamin D demand during pubertal maturation as vitamin D is required for rapid linear growth as well as bone accrual (24). As the finding indicated that vitamin D decreased in children >2 years old, it raised the question of whether children's vitamin D levels should be tested as early as 2 years old. A previous study with similar findings also highlighted the need for vitamin D supplementation in children >2 years old (25).

The high level of vitamin D in children under 2 years old might be related to, although was not explored in this study, dietary and behavioral aspects. The dietary intake in children under 2 years old differ from other age groups as in this age, breast milk is given. The World Health Organization recommended exclusive breastfeeding on the first 6 month of life, and continues up to 2 years old with appropriate complimentary food (26). The vitamin D level of breast milk remains rather steady even after prolonged breastfeeding. The breastmilk content is dependent on the level of vitamin D in the mother and, as a result, rises when breastfeeding women receive pharmaceutical vitamin D supplements (27). However, there has been a study showing that exclusively breastfeeding is a risk factor for vitamin D deficiency in children under 6 months old. The vitamin D

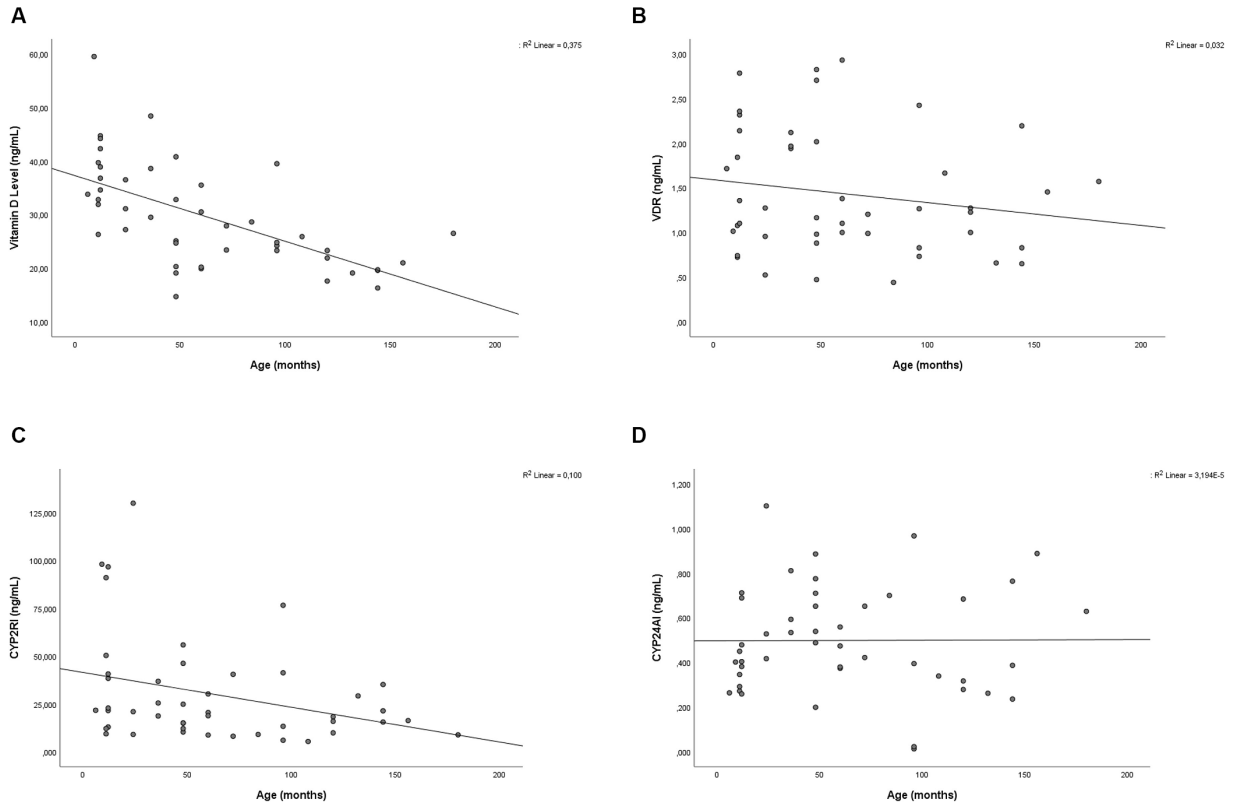

FIGURE 2
Scatter plot showing the relationship between age with **(A)** vitamin D, **(B)** VDR, **(C)** CYP2R1, and **(D)** CYP24A1 level.

TABLE 3 **Correlation analysis between vitamin D level, VDR, CYP2R1, CYP24A1, and subject characteristics.**

n = 46	Vitamin D (ng/mL)		VDR (ng/mL)		CYP2R1 (ng/mL)		CYP24A1 (ng/mL)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Sex	0.07	0.645	0.108	0.473	0.003	0.985	0.041	0.787
Age	−0.625	<0.001*	−0.14	0.354	−0.311	0.035*	−0.004	0.979
Vitamin D status	0.924	<0.001*	0.363	0.013*	0.259	0.083	−0.110	0.468
VDR	0.399	0.006*						
CYP2R1	0.185	0.219	−0.172	0.252				
CYP24A1	−0.103	0.496	−0.128	0.398	0.296	0.046*		

*$p < 0.05$ denotes statistical significance.

deficiency at birth in Indonesian infants by 6 months old, specifically among exclusively breastfed, was improved by the Indonesian culture of infant sunbathing (28). This study showed that with the increase of age, the vitamin D level decreased. It is in line with a previous study in Bahrain which indicated a negative correlation between vitamin D levels and age (20). Biological factors such as body composition shifting occur as children grow. Chen et al. found that in preschool children, the fat mass index decreased with age, as the fat-free mass index, consisting of skeletal muscle or lean mass, increased (29). Vitamin D is important to muscle regeneration, especially after muscle damage (30). In addition to inadequate vitamin D supplementation, the increased demand for muscle mass and fibers lowers the vitamin D level (31). Certain social and behavioral factors have been linked to vitamin D deficiency. In Southeast Asia, fair skin is considered the common beauty standard. These factors lead to sun avoidance behaviors including the usage of covered-up garments and the tendency of being indoors (6). Other behaviors that are related to lower levels of vitamin D due to lesser sun exposure include more indoor activities and increased screen time (9, 32, 33). VDR is a member of the steroid hormone receptor family and is involved in transcription in a variety of cell types. It can be found in the membrane, mitochondria, and nucleus of cells (34). Based on vitamin D status, VDR levels were significantly higher in the sufficient group in comparison to the insufficient group. Those who had sufficient vitamin D status had the highest VDR level. The relation of vitamin D, in particular calcitriol, and VDR has been established (2). The unbound VDR is mostly found in the nucleus and slightly distributed on the membrane (35). The distribution is related to VDR mechanism

TABLE 4 Multivariate analysis of vitamin D status factors.

	OR	95% CI		*p*-value	pseudo R^2
		Lower	Upper		
Parameter					0.620
Sex – male	0.549	0.120	2.520	0.441	
Age (years) – 0 to 2 years old	42.092	4.532	390.914	0.001*	
Age (years) – 2 to 6 years old	2.262	0.417	12.274	0.344	
VDR (ng/mL)	7.023	1.864	26.453	0.004*	
CYP2RI (ng/mL)	1.037	0.999	1.077	0.057	
CYP24AI (ng/mL)	0.149	0.006	3.716	0.246	

OR, odds ratio; CI, confidence interval; *$p < 0.05$ denotes statistical significance.

of work. The genomic effect is through the binding of the high-affinity intracellular VDR and calcitriol which forms a heterodimer with the retinoid X receptor. The target organs' essential gene expression is regulated as a result of the binding of heterodimer to vitamin D responsive elements (VDRE) in DNA once it reaches the cell nucleus (5). As VDR is essential in initiating vitamin D function, and vice versa, vitamin D is of paramount importance as it influences the VDR expression directly. Vitamin D has an impact on VDR upregulation by increasing VDR synthesis or decreasing receptor degradation (36, 37) Studies showed that vitamin D induced and stabilized the expression of VDR-messenger ribonucleic acid (mRNA) which led to the VDR level increment (38). The nongenomic effect signaling activation through the membrane bund VDR leads to MAP-kinase stimulation and involves interaction with ion channels, crosstalk with the nuclear VDR, and other transduction pathways. The non-genomic pathway provides a rapid response to the stimulus. However, the magnitude of the extracellular VDR effect still needs to be elucidated (39).

The current study showed that the increase in VDR levels is in line with vitamin D levels which is also depicted by the positive correlation between VDR and vitamin D status. It is supported by a previous randomized controlled trial that found vitamin D supplementation (2,000 IU/day) for 2 months increased the VDR gene expression 60 times ($p = 0.001$) (40). However, the VDR upregulation by vitamin D was suggested to be time and tissue-dependent (41). Furthermore, a systematic review and meta-analyses indicated that the response of the amount of vitamin D on VDR might be different in each individual due to genetic polymorphisms. For instance, TaqI and FokI polymorphisms have been associated with better responses to supplementation of vitamin D (42). Genetic, hormonal (PTH hormone, glucocorticoids, retinoic acid), epigenetic, and environmental factors (diet, sun exposure, pollution, and illness) have an impact on the control of VDR expression. Iriani et al. found that calcitriol increased the mRNA levels of the VDR gene (34). The inline progression of VDR and vitamin D level might be related to the negative regulation of vitamin D by membrane VDR. The membrane VDR works as the receptor of the rapid effect of $1,25(OH)_2D$ that induces a variety signaling pathways, for instance the pathway of phosphatidylinositol-3-kinase (PI3K), p21ras, Wnt5a, and phospholipase A_2 tyrosine kinase Src. Extracellular signal-regulated kinase 1/2 (ERK 1/2), PKC, JNK 1/2, PI3K, and p21ras rapid activation has been shown to regulate CYP24A1 induction by $1,25(OH)_2D$ (43).

The regulation of CYP2R1 is still poorly understood. There have been several factors linked to the CYP2R1 regulation. Clinical factors, including starvation, obesity, and diabetes, as well as genetic factors, for instance, mutation and polymorphism, were found to influence the expression of CYP2R1 (44). Roizen et al. found that aging significantly reduces the level of CYP2R1 in the liver of male mice, there has been no study indicating the correlation between CYP2R1 and age in pediatrics (45). In this study, age was found to be negatively associated with CYP2R1. The strength of the correlation test in both variables was weak, indicating the possibility of intermediate variables, for instance, vitamin D. This study also showed that vitamin D levels were lower in older children. Calcitriol suppression induced the expression of CYP2R1 mRNA in oral squamous cell carcinoma tumor cells. In the same study, treatment with calcitriol increased the expression of CYP2R1, proving the relationship between vitamin D and CYP2R1 level (46).

The two cytochromes, CYP2R1 and CYP24A1, pose a contracting effect on vitamin D levels. As CYP2R1 activates calcitriol, the CYP24A1 works as the catabolic enzyme (47, 48). Interestingly, a positive correlation was found between CYP2R1 and CYP24A1. The metabolism of vitamin D appears to be optimally regulated to prevent the synthesis of excess hormone and to break down the hormone or even its substrate by superinducing catabolic mechanisms such as CYP24A1 when needed (15). CYP24A1 is mainly affected by calcitriol, the active form of vitamin D which is first converted by CYP2R1. Calcitriol is known as a strong upregulation factor of CYP24A1 (44). CYP24A1 is not expressed in the absence of calcitriol. On the contrary, CYP24A1 is abundantly expressed in the kidney when calcitriol is detected to limit calcitriol production (49).

This study is the first to depict the association between vitamin D, VDR, CYP2R1, and CYP24A1 in Indonesian pediatrics. Geography and ethnicity are important factors in vitamin D metabolism as it is related to sun exposure intensity, the amount of melanin, and behavioral factors. In addition, the major strength of the current study is that we exclusively included healthy individuals. It is a sensible strategy to reduce variability by removing the impact of diseases on vitamin D levels, VDR, CYP2R1, and CYP24A1. Nevertheless, the current study used a cross-sectional design which does not reveal the causality between correlated factors. Moreover, this study did not include the status of lactation, diet, vitamin D supplementation, and the length of sun exposure of the subjects. Further studies with longitudinal design and inclusion of other variables are required to overcome this limitation.

Conclusion

In conclusion, our findings suggest that vitamin D level is reduced by the increase in age which suggests the need for vitamin D screening in children older than 2 years old. Vitamin D supplementation might benefit children in the adolescent age group. VDR level was also found to be significantly different based on Vitamin D status. Increasing vitamin D levels induces upregulation of VDR. Both vitamin D metabolism enzymes, CYP2R1 and CYP24A1, suggested inline-level progression.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Helsinki Declaration by the Ethics Commission of the Faculty of Medicine, YARSI University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

AI: Conceptualization, Formal analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. AR: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. MF: Formal analysis, Investigation, Writing – original draft. RG: Formal analysis, Investigation, Writing – original draft. AT: Formal analysis, Investigation, Writing – original draft. FM: Formal analysis, Visualization, Writing – original draft, Writing – review & editing. MN: Funding acquisition, Project administration, Resources, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Explorative case control study on the associations of serum vitamin D3, folic acid and vitamin B12 levels on Kawasaki disease and coronary artery lesions

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Background: Kawasaki Disease (KD) is a pediatric vasculitic disorder characterized by systemic small vasculitis, notably coronary arteritis, with unclear pathogenesis. This explorative case-control study investigated the association between folic acid (FA), vitamin D3 (VD3), and vitamin B12 (VB12) levels and the different types of Kawasaki Disease, as well as the incidence of coronary artery lesions (CALs).

Methods: In this explorative case control study, 365 KD children admitted to our hospital from January 1, 2022 to June 30, 2023 were included as the KD group. Simultaneously, 365 healthy children who received physical examination during the same period were included as the control group. The KD group was divided into typical KD group and incomplete KD group (IKD group), CALs group and non-CALS group, and IVIG sensitive group and IVIG resistant group. The children with CALs were divided into small tumor group, medium tumor group and large tumor group. Serum levels of FA, VB12, and VD3 were compared across all groups.

Results: Serum levels of FA and VD3 were significantly decreased in both the KD and CALs groups ($p < 0.05$), and both factors were identified as independent risk factors for KD and CALs. Similarly, reduced serum VD3 levels were observed in the IKD and IVIG-resistant groups ($p < 0.05$), with VD3 also being an independent risk factor for both IKD and IVIG resistance. Additionally, lower serum FA levels were noted in the group with large aneurysms ($p < 0.05$), establishing FA as an independent risk factor for aneurysm size.

Conclusion: Serum levels of folic FA and vitamin VD3 were significantly reduced in children with KD. Furthermore, these reductions were more pronounced in children with IKD and CALs. This pattern suggests that lower FA and VD3 levels may increase the risk of more severe coronary lesions in KD patients. Therefore, monitoring these biomarkers could provide valuable insights for early clinical diagnosis and intervention.

KEYWORDS

Kawasaki disease, coronary artery lesions, folic acid, vitamin D3, vitamin B12

1 Introduction

Kawasaki disease (KD) is a systemic vasculitic disorder commonly seen in pediatric clinics, primarily affecting children aged 6 months to 5 years (1). The primary pathological manifestation of KD is systemic vasculitis, with coronary arteritis being the predominant form. Notably, approximately 20% of KD patients develop complications involving coronary artery lesions (CALs) (2, 3). CALs secondary to KD are linked to alterations in specific biomarkers. These include counts of platelets and neutrophils, measures of plateletcrit and platelet distribution width, along with mean platelet volume, erythrocyte sedimentation rate, cardiac troponin I, endothelin-1, albumin, and hemoglobin levels (4). In a retrospective study involving 113 Chinese children, the Kobayashi score was applied to predict CALs in KD. Although the score demonstrated predictive capabilities, its accuracy was not sufficiently high. Therefore, there is a great need for reliable laboratory markers to accurately predict the subsequent development of CAL in the acute early stages of KD (5). The pathogenesis of KD is still not completely clear. It is currently believed that KD results from abnormal immune activation, which is induced by infections, especially in individuals with genetic susceptibility (4, 6). Previous study has shown that hyperhomocysteinemia can induce subclinical endothelial dysfunction, leading to advanced coronary arteritis in patients with KD (7). 5-methyltetrahydrofolate (5-MTHF) is the main circulating form of FA and is a methyl donor for the remethylation of homocysteine (Hcy) to methionine. FA other study has indicated that 10-methylenetetrahydrofolate reductase (MTHFR) is a candidate gene involved in the process of arteriosclerosis, and MTHFR is an enzyme that catalyzes the reduction of 5, 10-methyltetrahydrofolate to 5-MTHF (8). Therefore, it can be hypothesized that folic acid (FA) levels in the body may influence the development of arteriosclerosis. However, the specific role of FA in the progression of arteriosclerosis in KD patients remains unclear. Vitamin D3 (VD3) is an essential fat-soluble vitamin and a crucial steroid hormone. Its deficiency is widespread globally. Chen et al. (9) discovered that, besides regulating calcium and phosphorus metabolism, VD3 also influences immune disorders through the modulation of inflammatory signaling pathways. Reports suggested that adjunct therapy with 1-alpha, 25-dihydroxyvitamin D3, known for its anti-inflammatory and immunomodulatory properties, is beneficial in managing KD-induced vasculitis (10, 11). Comparatively, KD patients exhibit higher Vitamin D Receptor (VDR) expression in T cells than children with febrile respiratory infections or healthy individuals, suggesting that overactivated T cells may trigger the release of 25-(OH) D3. As a result, the enhanced inflammatory response observed in patients with CALs could result in an increased expression of VDR and consequently, elevated levels of 25-(OH) D3. Moreover, vitamin D deficiency has been recognized as an independent risk factor for arterial diseases (12). Therefore, the correlation between KD and VD3 has garneredincreasing attention. One study demonstrated that circulating 25-hydroxyvitamin D3 [25-(OH) D3] could predict CALs secondary to KD in children; however, the study was limited by a small sample size and low statistical power (13). VB12 is one of the most important B vitamins and serves as the primary coenzyme of Hcy forming methionine. The reduction of elevated homocysteine levels in the body depends on both FA and VB12, which provide methyl groups enabling the remethylation of homocysteine back to methionine, thus lowering Hcy levels (14). Many researchers' studies have demonstrated relations of high Hcy levels with low intake of FA and VB12 (15–18). FA and VB12 play important roles in remethylation and transsulfuration pathway of Hcy metabolism. A defect in any of these can lead to increased levels of homocysteine in circulation. Therefore, we hypothesized that FA, VD3, and VB12 levels are involved in the pathogenesis of KD and CALs. In this study, we used our data to conduct a preliminary exploration of the associations of FA, VB12, and VD3 on different types of KD and CALs.

2 Materials and methods

2.1 Object of study

In this prospective case-control study, 365 children with KD admitted to our hospital from January 1, 2022, to June 30, 2023 were selected as the KD group. Simultaneously, a total of 365 children from the same period were selected as the control group. The KD group was divided into typical KD ($n = 293$) and incomplete KD (IKD) ($n = 72$) groups. Based on their sensitivity to IVIG, they were divided into IVIG-sensitive ($n = 303$) and IVIG resistance groups ($n = 62$). Additionally, they were divided into CALs ($n = 18$) and non-CALs ($n = 347$) groups according to the presence of CALs, and the CALs group was further divided into small, medium, and large tumor groups according to the aneurysm size (see Table 1).

2.2 Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) a diagnosis of KD according to the 2017 American Heart Association (AHA) criteria; (2) all children in the KD group were diagnosed for the first time. (3) diagnostic criteria for coronary artery lesions (12): The Z-score was adopted as the diagnostic criterion for coronary artery lesions. (i) Normal: Z values < 2. (ii) Coronary artery dilation: $2 \leq Z$ value <2.5. (iii) Small coronary aneurysm: $2.5 \leq Z$ value < 5. (iv) Medium coronary aneurysm: $5 \leq Z$ value < 10, absolute diameter < 8 mm. (v) Large coronary aneurysm: Z-value < 10 or internal diameter > 8 mm. Z-value ≥ 2.0 indicates CAL+. (4) IVIG resistant KD is defined as patients with KD develop recrudescent or persistent fever ($T \geq 38°C$) at least 36 h after the end of IVIG infusion (2 g/kg).

TABLE 1 Distribution of samples.

Group	*n*	Gender (male%)	Age ($\bar{x} \pm s$)
KD	365	198 (54.25)	8.39 ± 0.62
Health group	365	188 (51.51)	8.85 ± 0.67
KD	293	160 (54.61)	8.28 ± 0.56
IKD	72	38 (52.78)	8.48 ± 0.61
IVIG-sensitive	303	164 (54.13)	8.17 ± 0.45
IVIG-resistance	62	36 (58.06)	8.22 ± 0.48
CALs group	18	11 (61.11)	8.83 ± 0.66
Non-CALs	347	148 (42.65)	8.49 ± 0.53
Small tumor	15	9 (60.00)	8.28 ± 0.55
Medium-large tumor	3	3 (100.00)	8.92 ± 0.74

The exclusion criteria for this study were as follows: (1) combined bacterial and viral infections; (2) combined cardiovascular and respiratory diseases; (3) use of glucocorticoids, immunosuppressants, and gamma globulin in the past 2 weeks. (4) Incomplete clinical data and unwillingness to participate with treatment.

2.3 Research method and observation index

Fasting venous blood (2 mL) was collected from all children and allowed to stand for 30 min before undergoing low-speed centrifugation at 2,500 rpm. After 15 min of centrifugation, supernatants were collected. Serum levels of FA (ng/ml), VD3 (VD3 refers to 1,25-dihydroxyvitamin D3, ng/ml), and VB12 (μg/l) FA were measured using an enzyme-linked immunosorbent assay (ELISA) purchased from Sigma. The levels of serum FA, VB12, and VD3 were also measured using a test kit from Shenzhen Teled Medical Co., Ltd., with an automatic chemiluminescence analyzer, VIT700.

2.4 Statistical approach

Statistical analyses were performed using SPSS software version 26.0. Measurement data that conformed to a normal distribution were presented as mean ± standard deviation, and analyzed using independent *T*-tests. Data not following a normal distribution were described using the median and interquartile range, and analyzed with the Mann–Whitney U test. Categorical data were expressed as percentages and assessed using the chi-square (χ^2) test or Fisher's exact test, as appropriate. Statistical significance was established at $p < 0.05$.

3 Results

3.1 Serum levels of FA, VB12, and VD3 among different groups

The KD group was divided into typical KD group and IKD group, CALs group and non-CALS group, IVIG sensitive group and IVIG resistant group, small tumor group, medium and large tumor group. Serum levels of FA, VB12, and VD3 were compared among all groups (see Table 2).

TABLE 2 Serum levels of FA, VB12, and VD3 among different groups ($\bar{x}\pm s$).

Group	*n*	FA (ng/ml)	VD (ng/ml)	WB12 (ug/l)
KD	365	9.82 ± 3.81	23.55 ± 7.85	465.93 ± 170.57
Health group	365	16.01 ± 7.07	56.38 ± 14.74	501.95 ± 177.41
KD	293	12.32 ± 4.58	40.44 ± 5.55	526.65 ± 126.12
IKD	72	11.58 ± 4.33	11.81 ± 3.86	518.83 ± 121.30
IVIG-sensitive	303	16.30 ± 6.89	26.12 ± 5.73	219.25 ± 80.34
IVIG-resistance	62	13.73 ± 8.12	11.02 ± 3.58	205.31 ± 79.71
CALs group	18	4.39 ± 5.80	6.61 ± 3.02	95.51 ± 26.88
Non-CALs	347	16.61 ± 6.60	25.03 ± 8.26	106.38 ± 27.23
Small tumor	15	5.15 ± 0.85	12.88 ± 4.54	105.61 ± 4.54
Medium-large tumor	3	0.60 ± 0.00	14.03 ± 4.71	100.00 ± 3.88

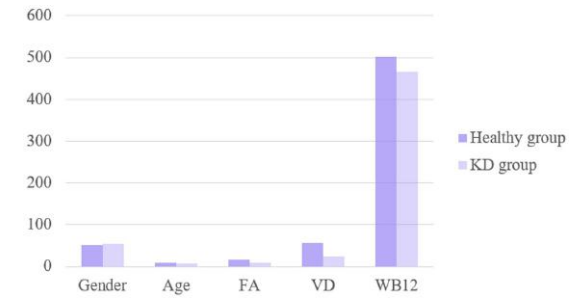

FIGURE 1
The levels of FA, VD and WB12 in healthy group and KD group were compared.

3.2 Comparisons of FA, VD3, and VB12 levels in healthy control group and KD group

There were no significant differences in sex or age between the two groups ($p > 0.05$). The levels of serum FA and VD3 indices in the KD group were significantly lower compared those in the healthy control group ($p < 0.05$), while there was no difference in the VB12 indices between the two groups, as shown in Figure 1. Multifactor regression analysis revealed that lower FA and VD3 indices are independent risk factors for KD, as detailed in Table 3.

TABLE 3 Multiple regression analysis.

Parameters	β	SE	Wald χ^2	OR	95%CI	*p*
FA	−1.155	0.413	7.874	3.176	1.415–7.123	0.004
VD3	−1.125	0.496	5.183	3.088	1.171–8.141	0.024
VB12	−1.334	0.290	7.992	3.345	1.515–6.995	0.089

FA, folic acid; VD3, vitamin D3; VB12, vitamin B12.

3.3 Comparisons of FA, VD3, and VB12 levels in typical and atypical KD groups

There were no significant differences in sex or age between the two groups ($p > 0.05$). Serum VD3 levels were significantly lower in the KD group compared to controls ($p < 0.05$); while there was no difference in the VB12 indices between the two groups, as shown in Figure 2. Multifactor regression analysis showed that the VD3 levels is an independent risk factor for IKD (Table 4).

3.4 The levels of FA, VD3, and VB12 were compared between IVIG-sensitive group and resistance group

There were no significant differences in the sex ratio, serum FA levels and VB12 levels between the two groups ($p > 0.05$). Serum vitamin D3 (VD3) levels were significantly higher in the IVIG-sensitive group

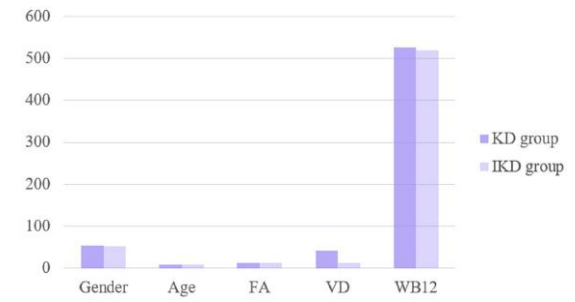

FIGURE 2
The levels of FA, VD and WB12 in KD and IKD groups were compared.

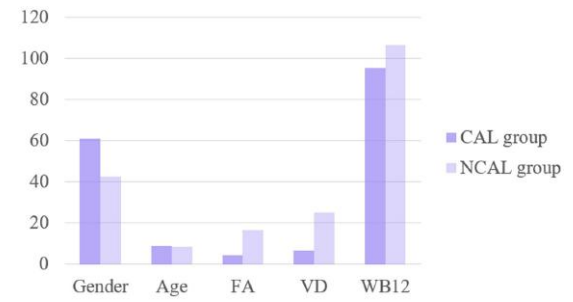

FIGURE 4
The levels of FA, VD and WB12 in the CAL group and the non-CAL group were compared.

TABLE 4 Multiple regression analysis.

Parameters	β	SE	Wald χ^2	OR	95%CI	*p*
VD3	1.184	0.344	7.628	3.918	1.525–7.324	0.002
VB12	1.557	0.401	10.032	4.643	1.326–8.243	0.075

VD3, vitamin D3; VB12, vitamin B12.

TABLE 6 Multiple regression analysis.

Parameters	β	SE	Wald χ^2	OR	95%CI	*p*
FA	−1.779	0.224	2.117	2.353	0.828–4.025	0.016
VD3	−1.617	0.277	3.736	1.475	0.356–3.053	0.031
VB12	−2.784	0.313	5.883	3.465	1.637–7.371	0.307

FA, folic acid; VD3, vitamin D3; VB12, vitamin B12.

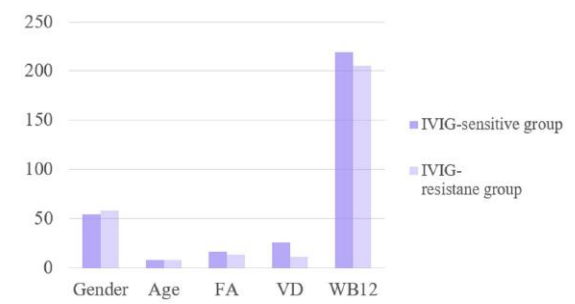

FIGURE 3
The levels of FA, VD and WB12 indexes were compared between IVIG-sensitive group and resistance group.

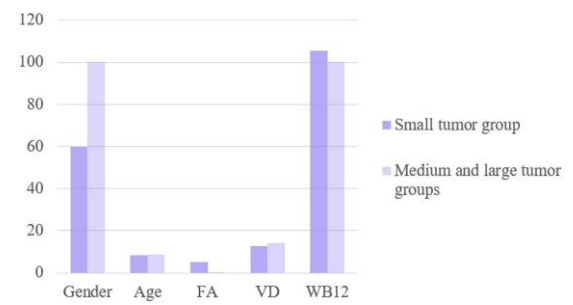

FIGURE 5
The levels of FA, VD and WB12 were compared between small tumor group and medium and large tumor groups.

TABLE 5 Multiple regression analysis.

Parameters	β	SE	Wald χ^2	OR	95%CI	*p*
VB12	1.295	0.335	4.886	2.875	1.202–5.062	0.135
VD3	0.937	0.293	3.133	1.593	0.859–3.686	0.016

VD3, vitamin D3; VB12, vitamin B12.

compared to the IVIG-resistant group ($p < 0.05$), as shown in Figure 3. Multifactor regression analysis showed that decreased serum VD3 level is an independent risk factor for IVIG resistance (Table 5).

3.5 Comparisons of FA, VD3, and VB12 levels in the CALs group and the non-CALs group

There were no significant differences in sex, age or VB12 levels between the two groups ($p > 0.05$). The serum FA and VD3 levels in the CALs group were significantly lower than those in the non-CALs group ($p < 0.05$), as shown in Figure 4. Multivariate regression analysis showed that FA and VD3 levels are independent risk factors for CALs (Table 6).

3.6 The levels of FA, VD3, and VB12 were compared between small tumor group and medium and large tumor groups

There were no significant differences in sex or age between the two groups ($p > 0.05$). The serum FA levels in the small tumor group were significantly higher than those in the medium and large tumor groups ($p < 0.05$), while there was no difference in the VD3 and VB12 levels among these groups ($p > 0.05$), as shown in Figure 5. Multivariate regression analysis showed that the FA level is an independent risk factor for aneurysm size (Table 7).

TABLE 7 Multiple regression analysis.

Parameters	β	SE	Wald χ^2	OR	95%CI	*p*
FA	−1.002	0.415	3.857	1.742	0.936–3.634	0.009
VB12	−2.373	0.332	5.693	2.216	1.114–5.291	0.552

FA, folic acid; VB12, vitamin B12.

4 Discussion

Consistent with previous studies, we observed that serum FA levels in the KD group were significantly lower than those of the healthy group. Specifically, a study conducted by the Japan Environment and Child Research Group (18) showed that insufficient FA intake in pregnant mothers is associated with the occurrence of KD in infants and that FA supplementation in pregnant mothers could reduce the risk of KD. FA serves an essential role as a cofactor in both nucleotide biosynthesis and methylation processes, emphasizing its importance in cellular function and development (19). In addition, Amarasekera et al. (20) reported a positive correlation between FA concentrations in neonatal cord blood and maternal blood. Maternal FA supplementation is associated with upregulation of DNA methylation and genomic imprinting in CD4+ progenitor cells. Furthermore, another study (21) showed that FA inhibited gene expression of inflammatory cytokines such as interleukin-1β, tumor necrosis factor-α, and monocyte chemotactic protein-1 in lipopolysaccharide-activated macrophages. Considering the significant role of these inflammatory cytokines in the pathophysiology of KD, we hypothesize that sufficient FA exposure *in utero* could influence the development of immune functions in offspring, potentially reducing the risk of developing KD. As there was no difference in the estimated daily dietary FA between the KD and healthy groups, further researches are needed to elucidate the mechanism by which FA prevents the onset of KD.

This study additionally revealed that serum FA levels in the IKD group were significantly lower than those in the KD group, and levels in the CALs group were significantly lower than in the non-CALs group. This has not been explicitly mentioned in previous studies, suggesting that FA levels are involved in the occurrence of CALs by influencing Hcy levels. Recent epidemiological studies indicated that Hcy levels are independent risk factors for coronary heart disease. Homocysteine, a sulfur-containing amino acid derived from methionine after demethylation, is synthesized with the assistance of methionine synthase, which requires VB12 as a cofactor (22). This study demonstrated that the serum FA levels in the small tumor group was significantly higher than that in the medium and large tumor groups, while VD3 and VB12 levels did not differ from the other two groups. This deviation from previous studies may be attributed to the limited sample size used in this study. The product of tetrahydrofolate catalyzed by N-5N-10-methylene tetrahydrofolate reductase (NTHFR) is the most important methyl donor *in vivo*. In addition, *in vivo* Hcy can condense with serine to form cystathione under the catalysis of cystathionine β-synthase (CBS) (23). When FA is deficient, the activities of NTHFR and CBS are significantly reduced, which leads to the obstruction of methionine regeneration and the excessive accumulation of Hcy. In conclusion, FA is an essential component for the conversion of Hcy to methionine and exhibits a significant negative correlation with Hcy levels. A deficiency in FA not only hampers the function of FA reductase but also disrupts the production of methyltetrahydrofolate, contributing to elevated Hcy levels.

The mechanisms by which homocysteine induces coronary artery lesions (CLAs) are complex, involving damage to the vascular endothelium, enhanced platelet activity, increased platelet aggregation, elevated fibrinogen production, and the promotion of smooth muscle cell migration, layering, and other pathophysiological processes (24, 25). These mechanisms bear a resemblance to the pathophysiological processes of CALs in KD; thus, it is hypothesized that homocysteine may also contribute to coronary artery injury in children with KD. Further studies (26) have demonstrated that homocysteine activates protein kinase C, which in turn promotes the expression of c-Fos and c-Myb in vascular endothelial and smooth muscle cells. Fundamental studies (27) have established a strong link between FA, a key factor in Hcy metabolism, and the development of CALs. In the realm of clinical research (28), findings indicated that Hcy levels were significantly higher in CHD patients than in non-CHD patients. Additionally, there is a negative correlation between FA levels and the incidence of CHD, with Hcy levels also showing a negative association with FA levels. However, FA is not an independent risk factor for CHD (29). It has been hypothesized that FA may influence homocysteine (Hcy) metabolism. Furthermore, a deficiency in FA leads to elevated Hcy levels, which, in turn, contributes to the development of CALs and CHD. This study confirmed significant differences in serum VD3 levels across groups: patients in the KD group exhibited lower VD3 levels compared to the healthy control group; within the KD cohort, those with IKD had significantly lower VD3 levels than those with complete KD; and patients with CALs had lower VD3 levels than those without CALs. Additionally, substantial evidence indicated that a deficiency in VD3 elevates the risk of cardiovascular diseases by impairing vascular endothelial function. Recent studies have shown that VD3 levels are related to KD, particularly KD combined with CALs. Research has demonstrated that serum levels of 25-(OH) D3 are significantly lower in children diagnosed with KD and that these levels are positively associated with the incidence of CALs and IKD (30). Furthermore, Stagi et al. (31) also found that a deficiency of serum 25 (OH) D3 in children with KD is closely related to an increased risk of cardiovascular events, and its low level may play an important role in the occurrence of KD combined with coronary complications. In addition, previous studies have found that the serum VD3 level of the population exhibit a seasonal pattern of being lower in winter and higher in summer (32), which is consistent with the seasonal variation trend of KD incidence. Thus, it is hypothesized that the seasonal pattern of KD incidence may be linked to the seasonal fluctuations in serum VD3 levels (33). However, the mechanism underlying the relationship between serum VD3 levels and the pathogenesis of KD remains unclear. Activation of immune factors and the release of inflammatory factors are increasingly recognized as critical elements in KD's pathogenesis. Experimental studies involving KD mice models have demonstrated overexpression of the transcription factor kappa B (κB) and matrix metalloproteinase-9 (MMP-9) (34). Other literatures also found that interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and other inflammatory factors are related to KD. Furthermore, cytokines such as TNF-α, IL-6, and IL-1β have been found to induce endothelial cells to increase the production of matrix metalloproteinases (MMPs) This action accelerates the breakdown of extracellular matrix proteins and basement membrane components, leading to the deterioration of vascular walls and promoting the occurrence of CALs (35). Studies in animal models and cell cultures have revealed that the loss of the VD3R gene can lead to higher levels of pro-inflammatory factors (including TNF-α, IL-6, and IL-β1) produced by bone marine-derived macrophages. Additionally,

the signaling pathway generated by the combination of VD3 and VD3R can inhibit the activation of NF-κB, which enhances the negative feedback regulation of the inflammatory response (36). VD3 deficiency leads to an unregulated and persistent inflammatory response, which may also explain the relationship between VD3 deficiency and inflammatory disorders, including KD, to a certain extent (37).

Our study also had some limitations. We measured only plasma folate levels, not its active form, 5-methyltetrahydrofolate (5-MTHF), which we plan to investigate further.

5 Conclusion

In summary, serum levels of FA and VD3 were reduced in children with KD. At the same time, it was found that the serum levels of FA and VD3 were particularly decreased in children with CALs, suggesting that the levels of FA and VD3 were involved in the occurrence and development of KD and CALs, providing a new research direction for the pathogenesis of KD and reducing the risk of CALs.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Kunming Children's Hospital, Kunming, Yunnan, China. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

YC: Writing – original draft, Writing – review & editing. XL: Writing – original draft, Writing – review & editing. BL: Formal Analysis, Investigation, Writing – review & editing. JL: Methodology, Writing – review & editing. LM: Conceptualization, Writing – review & editing. CY: Conceptualization, Writing – review & editing. LH: Conceptualization, Writing – review & editing. HL: Project administration, Writing – review & editing. LD: Methodology, Writing – review & editing. ZS: Writing – original draft, Writing – review & editing. XZ: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Associations of vitamin D status with all-cause and cause-specific mortality in long-term prescription opioid users

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Objective: This study aimed to investigate the association between serum 25-hydroxyvitamin D (25(OH)D) concentrations and mortality in long-term prescription opioid users.

Methods: The study included 1856 long-term prescription opioid users from the National Health and Nutrition Examination Survey (NHANES, 2001–2018). Mortality status were determined by matching with the National Death Index (NDI) records until December 31, 2019. Multivariable Cox proportional hazard models were constructed to assess the association.

Results: Over a median follow-up period of 7.75years, there were 443 cases of all-cause mortality, including 135 cardiovascular disease (CVD) deaths and 94 cancer deaths. After multivariable adjustment, participants with serum 25(OH)D concentrations within 50.00 to <75.00nmol/L and≥75nmol/L had a lower risk of all-cause mortality, with hazard ratios (HRs) of 0.50 (95% confidence interval [CI] 0.29, 0.86) and 0.54 (95% CI 0.32, 0.90), respectively. Nevertheless, no significant association was found between serum 25(OH)D concentrations and the risk of CVD or cancer mortality. The RCS analysis revealed a non-linear association of serum 25(OH)D concentration with all-cause mortality (p for non-linear=0.01). Per 1-unit increment in those with serum 25(OH)D concentrations <62.17nmol/L corresponded to a 2% reduction in the risk of all-cause mortality (95% CI 0.97, 1.00), but not changed significantly when 25(OH)D concentrations ≥62.17nmol/L.

Conclusion: In conclusion, a non-linear association existed between serum 25(OH)D concentrations and all-cause mortality in long-term prescription opioid users. Maintaining serum 25(OH)D concentrations ≥62.17 nmol/L may be beneficial in preventing all-cause mortality in this population.

KEYWORDS

prescription opioids, pain, mortality, NHANES, 25-hydroxyvitamin D

1 Introduction

Prescription opioids are widely recognized as the most commonly used and effective analgesics for managing moderate to severe pain, and the rates of prescription opioid use are increasing in many countries (1). Despite their significant pain-relieving properties, long-term use of prescription opioids increases the risk of overdose, misuse, addiction, and

other associated risks (2), with 16,706 fatalities attributed to prescription opioid overdose in 2021 (3). Increasing evidence suggests long-term prescription opioid use is associated with all-cause mortality (4–6). However, the results regarding the association between long-term prescription opioid use and cardiovascular or cancer mortality were contradictory. Ekholm et al. (6) reported no association was observed for long-term prescription opioid use with cardiovascular and cancer mortality. In contrast, Nalini et al. (7) found long-term opiate use was associated with an increased risk of cardiovascular mortality, and Song et al. (4) also observed a higher mortality rate due to cancer or circulatory system diseases in long-term opioid users.

Vitamin D is a steroid hormone, and 25-hydroxyvitamin D (25(OH)D) serves as the predominant circulating form of vitamin D in the bloodstream. Vitamin D in the body is primarily synthesized through the skin upon exposure to ultraviolet radiation, and it can also be obtained in small quantities from the diet (8). Apart from its role in maintaining bone health, vitamin D plays a crucial role in immune regulation, cell growth, and cellular differentiation (9). Several epidemiological studies have identified a link between low 25(OH)D concentrations and an increased risk of mortality in the general population (10, 11). Nevertheless, recent intervention studies have failed to demonstrate the benefit of vitamin D supplementation on mortality (12–14). Prior study have reported that chronic pain patients with vitamin D deficiency faced an increased risk of receiving higher opioid doses and using them for extended periods (15). Recent study have indicated vitamin D deficiency was associated with an increased risk of prescription opioid use and the exacerbation of opioid addiction (16). The absence of vitamin D signaling can increase sensitivity to morphine reward, leading to greater exogenous opioid consumption (16). Furthermore, vitamin D may exert analgesic effects through stimulating the body's anti-inflammatory response, scavenging reactive oxygen species, and regulating the endogenous opioid pathway, thereby affecting opioid use (17–19). Despite the presence of evidence indicates a connection between vitamin D deficiency and the use of prescription opioids, no reports have addressed the association of vitamin D deficiency with mortality in long-term prescription opioid users. Due to the variation in the impact of long-term prescription opioid use on mortality outcomes, further research is needed to elucidate the relationship between them.

To address this research gap, we conducted a prospective investigation to examine the associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality in long-term prescription opioid users. We hypothesized that low 25(OH)D concentrations would be associated with an increased risk of all-cause and cause-specific mortality in this population.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; NHANES, National Health and Nutrition Examination Survey; NCHS, National Center of Health Statistics; CDC, Centers for Disease Control and Prevention; ICD-10, International Classification of Diseases, 10th; NDI, National Death Index; PIR, poverty-income ratio; BMI, body mass index; MET, metabolic equivalent; CVD, cardiovascular disease; HbA1c, glycosylated hemoglobin; SE, standard errors; HR, hazard ratio; CI, confidence interval; RCS, restricted cubic spline.

2 Materials and methods

2.1 Study population

National Health and Nutrition Examination Survey (NHANES) is a nationally representative cross-sectional survey that utilizes a stratified multistage sampling design to evaluate the health and nutritional status of the non-institutionalized population in the United States (20). The research protocol was approved by the Institutional Review Board of the National Center for Health Statistics (NCHS), and written informed consent was obtained from all participants. Since the de-identified data analyzed during the current study were publicly available from NHANES, the study did not require any review board approval again.

This study included a total of 50,201 participants aged ≥20 years from 2001 to 2018. We excluded non-long-term prescription opioid users ($n = 47{,}426$), individuals receiving medications for opioid dependence or withdrawal (including buprenorphine; naloxone, buprenorphine; methadone, $n = 79$), those with missing vitamin D data ($n = 331$), those with missing follow-up data ($n = 1$), and those with missing covariate data ($n = 508$). Ultimately, the analysis included 1856 participants. The participant inclusion process was detailed in Supplementary Figure S1.

2.2 Measurement of serum 25(OH)D concentrations

Before 2005–2006, serum 25(OH)D concentrations were measured using the DiaSorin RIA kit (Stillwater, MN, USA). Since 2005–2006, a standardized liquid chromatography–tandem mass spectrometry (LC–MS/MS) method had been used to measure serum 25(OH)D concentrations. Following the analytical guidelines of Centers for Disease Control and Prevention (CDC), serum 25(OH)D concentrations from 2005 to 2006 and earlier were converted to equivalent concentrations from LC–MS/MS using regression, and LC–MS/MS equivalent data were utilized in all analyses.

2.3 Ascertainment of long-term prescription opioid users

During the household interview survey, participants were asked if they had taken a medication in the past month for which they needed a prescription. Participants who answered "yes" were asked to report the names of the medications through medication containers, verbal reports, or pharmacy receipts. Additionally, participants were also asked how long they had been taking the medication. All recorded medications were classified using the Multum Lexicon therapeutic classification system. Participants who reported the use of narcotic analgesics or narcotic analgesic combinations were considered as prescription opioid users, and the duration of prescription opioid use ≥90 days was considered as long-term use (21). Methadone, naloxone, and buprenorphine were often used to treat opioid dependence or withdrawal, we exclude participants taking these drugs from prescription opioid users.

2.4 Ascertainment of mortality

Mortality status and follow-up time were determined by matching with the National Death Index (NDI) records available until December 31, 2019. According to the International Classification of Diseases, 10th Revision (ICD-10), cardiovascular disease-specific mortality was defined as deaths due to heart disease (I00-I09, I11, I13, I20-I51) or cerebrovascular disease (I60-I6), while cancer-specific mortality was defined as deaths due to malignant neoplasms (C00-C97).

2.5 Covariates

Sociodemographic information [age, sex, race/ethnicity, education, poverty-income ratio (PIR)], living habits (physical activity, cotinine, alcohol consumption), comorbidity (history of hypertension, cardiovascular disease (CVD), diabetes, and cancer), as well as body mass index (BMI), were obtained from NHANES. Race/ethnicity was categorized as Non-Hispanic White, Non-Hispanic Black, Mexican American, and Other. Education was divided into less than high school, high school, and more than high school. PIR was classified as ≤130, 130–300%, and > 300% (22). Physical activity was calculated as weekly minutes of moderate and vigorous activity multiplied by metabolic equivalent (MET) levels. Alcohol consumption was categorized as never (<12 drinks in a lifetime), former (≥12 drinks in a year or ≥ 12 drinks in a lifetime but none in the past year), mild (female: <2 drinks/d, male: <3 drinks/d), moderate (female: 2 to <3 drinks/d, male: 3 to <4 drinks/d, or 2–4 binges/month), and heavy (female: ≥3 drinks/d, male: ≥4 drinks/d, or ≥ 5 binges/month) (23). BMI was calculated as weight (kilograms) divided by the square of height (meters) and classified as <25, 25–30, and ≥ 30 (24). Hypertension was assessed by systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 80 mmHg, or taking antihypertensive drugs (25). Diabetes was determined by self-reported diagnosis by doctor or glycosylated hemoglobin (HbA1c) level ≥ 6.5% or taking anti-diabetic drugs. CVD was confirmed through self-reported diseases, including coronary heart disease, congestive heart failure, heart attack, stroke, and angina.

2.6 Statistical analyses

All analyses were conducted using appropriate sample weights to account for the complex sampling design of NHANES. Serum 25(OH)D concentrations were classified into four groups: severe deficiency (<25.00 nmoL/L), deficiency (25.00 to <50.00 nmoL/L), insufficiency (50.00 to <75.00 nmoL/L), and sufficiency (≥75.00 nmoL/L) (26). The reference group was defined as those with serum 25(OH)D concentrations <25.00 nmoL/L. Continuous variables and categorical variables were presented as means (standard errors, SE) and frequencies (weighted percentages), respectively. Differences between the four 25(OH)D groups were assessed using ANOVA for continuous variables and chi-square test for categorical variables. Multivariable Cox proportional hazards regression models were created to investigate the association between serum 25(OH)D concentrations and risks of all-cause or cause-specific mortality. Model 1 made no adjustments; model 2 was adjusted for age, sex, race/ethnicity; model 3 was further adjusted for education, alcohol consumption, cotinine, physical activity, BMI, PIR, hypertension, diabetes, cancer, and CVD. Restricted cubic spline analysis (RCS) with 5 knots (5th, 28th, 50th, 73th, and 95th) was applied to examine the non-linear relationship between serum 25(OH)D concentrations and all-cause mortality. Stratified analyses were further performed by age (<60 or ≥ 60 years), sex (male or female), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American, and Other), BMI (< 25, 25–30, and ≥ 30), and cancer (yes or no). We also conducted several sensitivity analyses: (1) Participants who died within 2 years of follow-up were excluded to minimize reverse causality; (2) Participants with a history of cardiovascular disease or cancer were further excluded; (3) Additionally, adjustments were made for the blood drawing season to account for the influence of seasonal variations on vitamin D status.

All analyses were conducted using R 4.2.3 (Boston, MA, USA), and a two-tailed p-value <0.05 was considered statistically significant.

3 Results

3.1 Characteristics of participants

Of the 1856 participants with long-term prescription opioid use (mean [SE] age, 54.12 (0.5) years; 808 male [weighted, 40.7%]; 1,060 [weighted, 78.5%] non-Hispanic White; mean [SE] serum 25(OH)D concentrations, 70.19 (1.2) nmol/l), 32.4% were with deficient vitamin D (<50 nmoL/L), and 67.2% were with insufficient vitamin D (<75.00 nmoL/L). Table 1 shows the baseline characteristics of the study participants stratified by serum 25(OH)D concentrations. Compared to sufficient vitamin D (≥75.00 nmoL/L), the age of participants with severe deficient vitamin D (<25.00 nmoL/L) was younger. Severe deficient vitamin D (<25.00 nmoL/L) was more likely to occur in participants with of female, Non-Hispanic Black and Mexican American, with less physical activity, with obesity or history of CVD and diabetes. In addition, participants with severe deficient vitamin D were less likely to have higher PIR (>300%), have history of cancer, and have mild alcohol consumption.

During a median follow-up of 7.75 years, we identified 443 all-cause deaths (weighted, 18.7%), including 135 CVD deaths (weighted, 4.9%), and 94 cancer deaths (weighted, 3.8%).

3.2 Associations of serum 25(OH)D concentrations and mortality in long-term prescription opioid users

Table 2 displays the results of Cox proportional hazards regression analyses between serum 25(OH)D concentrations and mortality. After multivariable adjustment (model3), compared to participants with serum 25(OH)D concentrations <25.00 nmol/L, the hazard ratio (HR) for all-cause mortality for those with serum 25(OH)D concentrations ranging from 50.00 to <75.00 nmol/L was 0.50 (95% confidence interval [CI] 0.29, 0.86), for those with serum 25(OH)D concentrations ≥75 nmol/L was 0.54 (95% CI 0.32, 0.90). Nevertheless, no significant inverse associations were observed for serum 25(OH)D concentrations with CVD-specific mortality and cancer-specific mortality after multivariable adjustment. It is important to note that there was no

TABLE 1 Baseline characteristics of long-term prescription opioid users in NHANES, 2001–2018.

Characteristics	Serum 25(OH)D (nmol/l)					
	Total	<25.00	25.00 to < 50.00	50.00 to < 75.00	≥75.00	*p*-value
NO. (%)	1856	101 (5.4)	502 (27.0)	644 (34.7)	609 (32.8)	
Age, mean (SE), y	54.12 (0.5)	53.73 (1.6)	51.76 (0.7)	52.87 (0.7)	56.59 (0.8)	< 0.001
Cotinine, mean (SE), ng/ml*	95.34 (5.5)	105.40 (16.2)	117.04 (11.6)	94.04 (8.4)	83.70 (7.6)	0.07
PA, mean (SE), MET/week	2050.47 (147.2)	1246.69 (410.0)	1433.13 (147.9)	2033.91 (202.5)	2473.86 (293.6)	0.003
Sex, male, *n* (%)	808 (40.7)	38 (27.5)	216 (38.4)	297 (45.5)	257 (38.8)	0.02
Race/ethnicity, *n* (%)						< 0.001
Non-Hispanic White	1,060 (78.5)	26 (44.2)	204 (63.2)	389 (79.2)	441 (89.1)	
Non-Hispanic Black	382 (9.7)	50 (40.1)	152 (18.6)	107 (7.4)	73 (4.2)	
Mexican American	205 (4.4)	15 (8.7)	79 (8.1)	69 (4.1)	42 (2.3)	
Other	209 (7.5)	10 (7.0)	67 (10.1)	79 (9.3)	53 (4.4)	
PIR, *n* (%)						< 0.001
≤130%	809 (30.2)	42 (34.9)	252 (40.2)	291 (31.9)	224 (22.7)	
130–300%	569 (31.0)	40 (44.1)	138 (29.0)	189 (30.1)	202 (31.7)	
>300%	478 (38.9)	19 (21.0)	112 (30.8)	164 (38.0)	183 (45.6)	
Education, *n* (%)						0.13
Less than high school	186 (5.1)	11 (6.5)	55 (6.3)	69 (5.8)	51 (3.6)	
High school	824 (42.0)	45 (40.1)	241 (46.7)	290 (41.8)	248 (39.7)	
More than high school	846 (53.0)	45 (53.4)	206 (47.0)	285 (52.4)	310 (56.7)	
BMI, kg/m^2, *n* (%)						< 0.001
< 25	396 (22.5)	19 (13.6)	83 (17.6)	135 (22.6)	159 (26.0)	
25–30	540 (31.1)	17 (14.5)	124 (25.3)	196 (31.0)	203 (35.8)	
≥ 30	920 (46.4)	65 (71.9)	295 (57.1)	313 (46.4)	247 (38.2)	
Alcohol consumption, *n* (%)						0.004
Never	203 (7.6)	19 (15.6)	58 (9.5)	61 (5.8)	65 (7.5)	
Former	557 (26.7)	30 (32.9)	159 (29.9)	194 (27.5)	174 (23.6)	
Mild	557 (32.9)	19 (17.6)	150 (29.9)	194 (32.4)	194 (36.3)	
Moderate	246 (17.0)	14 (14.9)	52 (12.4)	89 (17.0)	91 (19.8)	
Heavy	293 (15.8)	19 (19.0)	83 (18.4)	106 (17.4)	85 (12.7)	
Hypertension, *n* (%)	1,352 (68.3)	77 (76.3)	367 (70.1)	455 (65.5)	453 (69.1)	0.30
CVD, *n* (%)	467 (19.7)	34 (36.9)	139 (25.0)	143 (16.3)	151 (18.5)	< 0.001
Diabetes, *n* (%)	526 (21.6)	47 (46.7)	162 (27.0)	163 (19.6)	154 (18.3)	< 0.001
Cancer, *n* (%)	320 (18.4)	12 (12.3)	63 (15.4)	106 (16.5)	139 (22.2)	0.03

NHANES, National Health and Nutrition Examination Survey; SE, standard error; MET, metabolic equivalent; PIR, poverty-income ratio; PA, physical activity; BMI, body mass index; CVD, cardiovascular disease; 25(OH)D, 25-hydroxyvitamin D.
*SI conversion factor: to convert cotinine to nanomoles per liter, multiply by 5.675.
All estimates accounted for survey weights; *p*-values for continuous variables and categorical variables were calculated with one-way ANOVA and chi-square test, respectively.

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TABLE 2 HRs (95% CI) for mortality risk according to serum 25(OH)D concentrations in long-term prescription opioid users in NHANES, 2001–2018.

	Serum 25(OH)D (nmol/l)				*p* for trend
	<25.00	25.00 to < 50.00	50.00 to < 75.00	≥75.00	
All-cause mortality					
No. of deaths (%)	36 (32.9%)	123 (22.1%)	160 (17.7%)	124 (16.4%)	
Model 1	1.00 (reference)	0.54 (0.33, 0.90)	0.47 (0.30, 0.74)	0.56 (0.36, 0.88)	0.38
Model 2	1.00 (reference)	0.54 (0.33, 0.88)	0.41 (0.25, 0.66)	0.42 (0.27, 0.67)	0.02
Model 3	1.00 (reference)	0.58 (0.34, 1.01)	0.50 (0.29, 0.86)	0.54 (0.32, 0.90)	0.19
CVD-specific mortality					
No. of deaths (%)	16 (13.5%)	36 (5.9%)	48 (4.2%)	35 (4.3%)	
Model 1	1.00 (reference)	0.34 (0.16, 0.71)	0.26 (0.12, 0.56)	0.33 (0.15, 0.72)	0.22
Model 2	1.00 (reference)	0.41 (0.19, 0.89)	0.30 (0.13, 0.66)	0.28 (0.12, 0.66)	0.02
Model 3	1.00 (reference)	0.57 (0.26, 1.24)	0.49 (0.21, 1.12)	0.49 (0.21, 1.12)	0.19
Cancer-specific mortality					
No. of deaths (%)	4 (4.3%)	25 (4.0%)	40 (4.9%)	25 (2.8%)	
Model 1	1.00 (reference)	0.73 (0.19, 2.76)	0.92 (0.22, 3.81)	0.67 (0.17, 2.56)	0.66
Model 2	1.00 (reference)	0.79 (0.18, 3.38)	0.78 (0.16, 3.79)	0.45 (0.10, 2.01)	0.10
Model 3	1.00 (reference)	0.68 (0.13, 3.60)	0.75 (0.12, 4.92)	0.46 (0.08, 2.65)	0.21

SI conversion factor: to convert cotinine to nanomoles per liter, multiply by 5.675.
Model 1: unadjusted. Model 2: adjusted for age, sex, race/ethnicity. Model 3: further adjusted for education, alcohol consumption, cotinine, physical activity, BMI, PIR, hypertension, diabetes, cancer, CVD.
NHANES, National Health and Nutrition Examination Survey; HR, hazard ratio; CI, confidence interval; PIR, poverty-income ratio; BMI, body mass index; CVD, cardiovascular disease; 25(OH)D, 25-hydroxyvitamin D.

linear association between serum 25(OH)D concentrations and all-cause mortality (p for trend = 0.19).

3.3 Dose–response relationship of serum 25(OH)D concentrations and all-cause mortality in long-term prescription opioid users

We used the RCS curve to estimate the dose–response relationship between serum 25(OH)D concentrations and all-cause mortality, and a non-linear association was observed after adjusting all confounders (p for non-linear = 0.01) (Figure 1). The inflection points for serum 25(OH)D concentrations were 62.17 nmoL/L and 94.52 nmol/L. Subsequently, piecewise COX proportional hazards regression was performed to analyze a threshold effect of serum 25(OH)D concentrations on all-cause mortality (Table 3). When serum 25(OH)D concentrations were < 62.17 nmoL/L, per 1-unit increment in serum 25(OH)D concentrations, there was a 2% lower risk of all-cause mortality (HR 0.98, 95%CI [0.97, 1.00]). However, there was no benefit effect for lower risk of all-cause mortality when elevate serum 25(OH)D concentrations for participants with serum 25(OH)D concentrations were 62.17- <94.52 nmol/L (HR 1.01, 95%CI [0.98, 1.03]) or ≥ 94.52 nmol/L (HR 0.97, 95%CI [0.93, 1.02]).

3.4 Stratified and sensitivity analyses

In stratified analyses, the benefit of high serum 25(OH)D concentrations (≥ 62.17 nmol/L) and low serum 25(OH)D concentrations (< 62.17 nmol/L) for the survival rate of participants with long-term prescription opioid use stratified by age, sex, race/ethnicity, BMI, and cancer was similar. No modification factors were found between serum 25(OH)D concentrations and these stratified variables (all p for interaction >0.05) (Figure 2). In sensitivity analyses, the results were also typically robust when excluding deaths that occurred within 2 years (Supplementary Table S1); excluding participants with history of CVD or cancer (Supplementary Table S2); further adjustment of blood drawing season (Supplementary Table S3).

4 Discussion

In this prospective cohort study of participants with long-term prescription opioid use, we discovered significantly non-linear inverse association between serum 25(OH)D concentrations and all-cause mortality, nevertheless, no inverse associations were observed for serum 25(OH)D concentrations with CVD-specific mortality and cancer-specific mortality. Lower vitamin D in participants with serum 25(OH)D concentrations < 62.17 nmol/L were associated with an increased risk of all-cause mortality. Stratified and sensitivity analyses supported the stability of our conclusions.

Our research revealed that a significant proportion (67.2%) of long-term prescription opioid users had insufficient serum 25(OH)D concentrations, highlighting a prevalent vitamin D deficiency within this population. Preclinical study have demonstrated that vitamin D deficiency accelerated the development of opioid tolerance and exacerbated opioid dependence, resulting in elevated

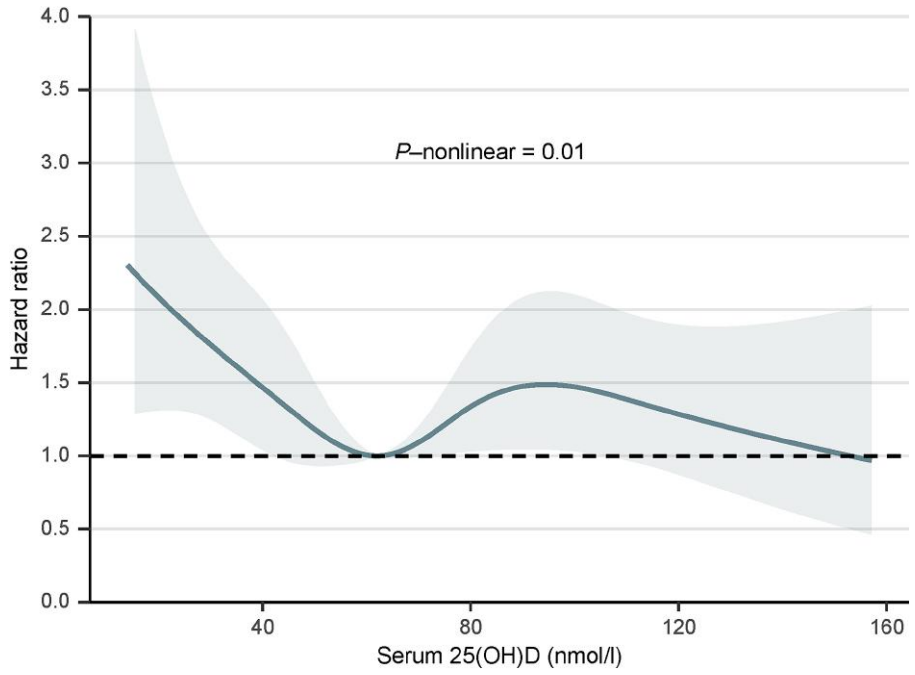

FIGURE 1
Dose–response relationship between serum 25(OH)D concentrations and all-cause mortality, 2001 to 2018. The model was adjusted for age, sex, race/ethnicity, education, alcohol consumption, cotinine, physical activity, BMI, PIR, hypertension, diabetes, cancer, and CVD. SI conversion factor: to convert cotinine to nanomoles per liter, multiply by 5.675. PIR, poverty-income ratio; BMI, body mass index; CVD, cardiovascular disease; 25(OH)D, 25-hydroxyvitamin D.

TABLE 3 Threshold effect analysis of serum 25(OH)D concentrations on all-cause mortality in long-term prescription opioid users in NHANES, 2001–2018.

All-cause mortality	Adjusted HR (95% CI), *p*-value
Inflection point	62.17 nmoL/L, 94.52 nmol/L
Per 1-unit increment in 25(OH)D concentrations <62.17 nmol/L	0.98 (0.97, 1.00), 0.02
Per 1-unit increment in 25(OH)D concentrations 62.17- < 94.52 nmol/L	1.01 (0.98,1.03), 0.57
Per 1-unit increment in 25(OH)D concentrations ≥94.52 nmol/L	0.97 (0.93,1.02), 0.22

Adjusted for age, sex, race/ethnicity, education, alcohol consumption, cotinine, physical activity, BMI, PIR, hypertension, diabetes, cancer, CVD.
NHANES, National Health and Nutrition Examination Survey; HR, hazard ratio; CI, confidence interval; PIR, poverty-income ratio; BMI, body mass index; CVD, cardiovascular disease; 25(OH)D, 25-hydroxyvitamin D.

opioid consumption (16). Turner et al. (15) have reported that chronic pain patients with vitamin D deficiency exhibited significantly greater prescription opioid doses and prolonged duration of use. Excessive or long-term use of prescription opioids undoubtedly increases the risk of mortality. Consequently, there is a basis to hypothesize that vitamin D deficiency heightens the mortality risk in long-term prescription opioid users. Prior epidemiological studies have indicated the association between low serum 25(OH)D concentrations and increased all-cause mortality risk within the general population or specific subgroups (27–31), aligning with our findings. The majority of studies have demonstrated that low serum 25(OH)D concentrations are associated with increased incidence of cardiovascular events and cardiovascular mortality (32–34). Nevertheless, our study did not observe a relationship between low serum 25(OH)D concentrations and the risk of cardiovascular mortality, potentially attributable to the focus on specific high-risk population, a smaller sample size, and differences in ethnicity. Likewise, we did not detect low serum 25(OH)D concentrations linked to an increased risk of cancer mortality. The association between vitamin D status and cancer mortality was controversial (35). A meta-analysis conducted by Chowdhury et al. (36) proposed that elevated baseline serum 25(OH)D concentrations were linked to a decreased risk of cancer mortality. Conversely, several other extensive prospective studies failed to identify an association between serum 25(OH)D concentrations and the risk of cancer mortality (37–39).

In long-term prescription opioid users, we observed a non-linear relationship between serum 25(OH)D concentrations

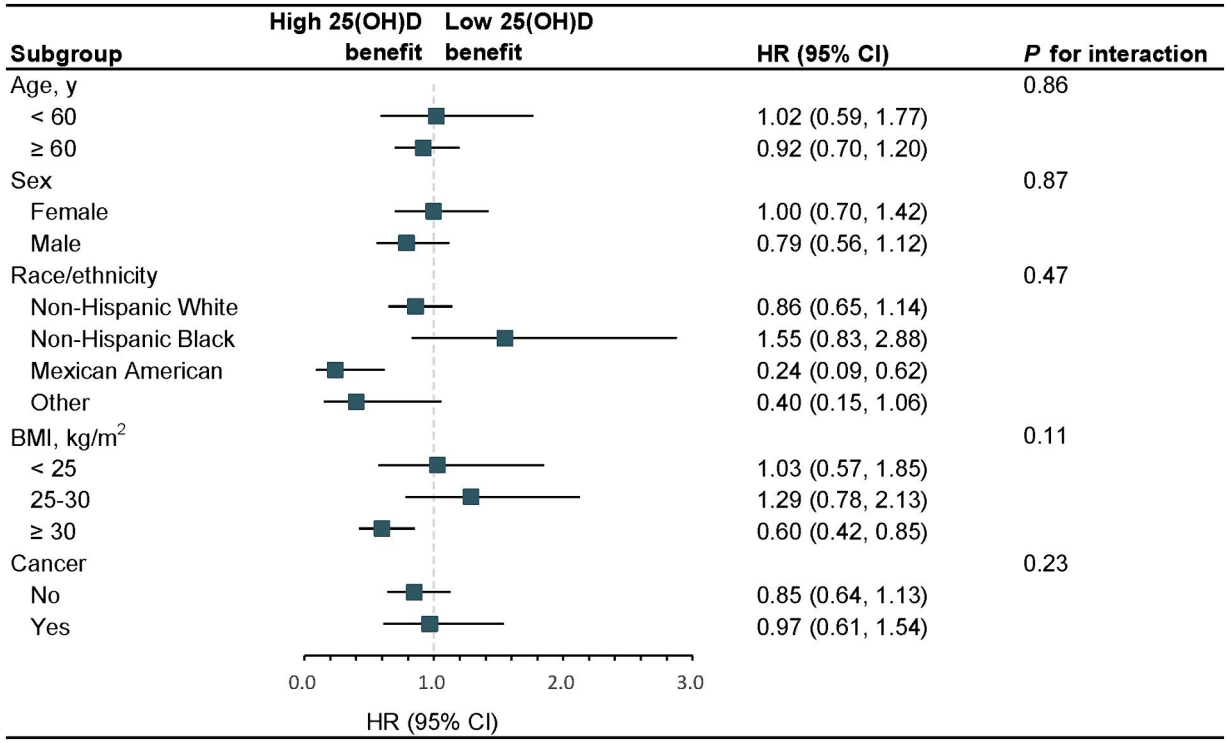

FIGURE 2
Stratified analyses of serum 25(OH)D concentrations with the risk of all-cause mortality, 2001–2018. The model was adjusted for age, sex, race/ethnicity, education, alcohol consumption, cotinine, physical activity, BMI, PIR, hypertension, diabetes, cancer, and CVD. The strata variable was not included in the adjustment when stratifying by itself. SI conversion factor: to convert cotinine to nanomoles per liter, multiply by 5.675. HR, hazard ratio; CI, confidence interval; PIR, poverty-income ratio; BMI, body mass index; CVD, cardiovascular disease; 25(OH)D, 25-hydroxyvitamin D.

and the risk of all-cause mortality (27, 40, 41), aligning with earlier findings in diverse populations. In comparison to the optimal serum 25(OH)D concentrations reported in other populations, our study revealed that sustaining serum 25(OH)D concentrations above 62.17 nmol/L corresponded to a decreased risk of all-cause mortality in long-term prescription opioid users. Collectively, these studies indicate the presence of a ceiling effect for serum 25(OH)D concentrations concerning health outcomes, including mortality, wherein surpassing a specific threshold may not yield additional advantages. Although consensus regarding the ideal serum 25(OH)D concentrations is lacking, the National Institutes of Health advised maintaining levels above 50.00 ng/mL to mitigate the health hazards linked to vitamin D insufficiency (42). Despite observational studies suggest that low serum 25(OH)D concentrations contribute to mortality risk, recent intervention studies have failed to demonstrate the advantages of vitamin D supplementation (12–14). Likewise, Neale et al. (43) observed that monthly vitamin D supplementation in the elderly did not result in lower all-cause mortality. A meta-analysis conducted by Zhang et al. (44) indicated that vitamin D supplementation alone only reduced cancer mortality in the general population. Wu et al. (45) discovered that monthly vitamin D supplementation did not lead to a reduction in prescription opioid use. However, these experiments included participants with high baseline serum 25(OH)D concentrations, such as 115 nmol/L in Neale RE et al.'s study and 66.4 nmol/L in Wu et al.'s study (exceeding 50 nmol/L), thereby constraining the efficacy of supplementation tests within the low serum 25(OH)D concentration subgroups. Subsequent studies exploring the potential of vitamin D supplements to mitigate all-cause mortality in long-term prescription opioid users should consider enrolling participants with lower baseline serum 25(OH)D concentrations.

This study possesses several strengths. Firstly, to the best of our knowledge, this was the first study to investigate the relationship between vitamin D deficiency and mortality in long-term prescription opioid users. Secondly, this study relied on a nationally representative sample and exhibited excellent follow-up rates. Lastly, several sensitivity analyses were performed to affirm the model's stability, thereby bolstering the reliability of our study's findings. Nonetheless, this study possesses certain limitations. Firstly, owing to its nature of cross-sectional design, it was unable to establish a causal relationship between vitamin D deficiency and mortality. Then, despite numerous potential confounding factors had been adjusted for, residual confounding factors might persist. Additionally, the absence of repeated measurements of serum 25(OH)D concentrations precluded the evaluation of the influence of dynamic fluctuations in vitamin D status on mortality.

5 Conclusion

In conclusion, a non-linear association was observed between serum 25(OH)D concentrations and all-cause mortality in long-term prescription opioid users. Lower vitamin D in participants with serum

25(OH)D concentrations <62.17 nmol/L were associated with an increased risk of all-cause mortality.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: http://www.cdc.gov/nchs/nhanes.htm.

Ethics statement

The studies involving humans were approved by NCHS Ethics Review Board, National Center for Health Services. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SD: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. JW: Data curation, Investigation, Writing – review & editing. PW: Data curation, Validation, Visualization, Writing – review & editing. ZH: Conceptualization, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1422084/full#supplementary-material

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Factors associated with vitamin D deficiency in health care workers exposed to SARS-CoV-2: a cross-sectional study

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Introduction: Globally, up to 76.6% of the population may be affected by vitamin D (VD) deficiency, which has been linked to increased morbidity and mortality from COVID-19. This underscores the importance of further research into VD supplementation, particularly for health care workers, who are at higher risk due to indoor work environments and dietary challenges associated with shift schedules.

Objective: This study aimed to identify factors associated with VD deficiency in Mexican health care workers exposed to SARS-CoV-2.

Materials and methods: We conducted a cross-sectional study from June 2020 to January 2021 among frontline health care workers treating hospitalized COVID-19 patients. Blood samples were collected to measure 25-hydroxy VD levels via radioimmunoassay. We also assessed previous COVID-19 infection and comorbidities that could influence VD levels.

Results: The study included 468 health care workers. The median serum VD concentration was 16.6 ng/mL. VD deficiency was found in 69.4% ($n = 325$) of participants, while only 5.1% ($n = 24$) had normal levels. Those with type 2 diabetes (13.3 ng/mL vs. 17.1 ng/mL) or obesity (15.7 ng/mL vs. 17.1 ng/mL) had significantly lower VD levels than their counterparts ($p < 0.001$ and $p = 0.049$, respectively). No significant differences were found among participants with high blood pressure. Multivariate analysis revealed that type 2 diabetes was independently associated with VD deficiency.

Conclusion: There is a high prevalence of VD deficiency among health care workers, which is potentially linked to both personal health factors and occupational conditions.

KEYWORDS

25-hydroxy vitamin D, vitamin D deficiency, health care worker, obesity, type 2 diabetes

Introduction

Vitamin D (VD) deficiency poses a significant global health challenge, with research indicating a prevalence rate of up to 76.6%, underscoring substantial regional variability and the impact of diverse determinant factors (1). Certain occupational groups exhibit a heightened prevalence of VD deficiency. This issue is partly linked to limited sun exposure inherent to specific job roles, particularly those conducted predominantly indoors or within shift work schedules, including night shifts (2). Additionally, those working night or rotating shifts are prone to less healthy dietary patterns. This tendency is influenced by a lack of suitable nutritional options and restricted eating times due to long working hours, leading to diets high in ultra-processed foods, meats, fats, and alcoholic beverages (3). Such dietary habits, combined with the demands of shift work, may contribute to the development of digestive disorders, sleep disturbances, and comorbidities (4). These risk factors, including limited sun exposure, inadequate diet, and obesity, which are inherent to the health care profession, significantly contribute to VD deficiency (5, 6).

During the COVID-19 pandemic, populations in areas with a high prevalence of VD deficiency experienced increased morbidity and mortality related to COVID-19 infection. This finding supports further studies on the prevalence of VD deficiency and the benefits of VD supplementation across various pathologies, from cardiometabolic disorders to viral infections (7). During the pandemic, the working conditions of health care workers worsened, likely due to the increase in indoor activities—both work-related and personal—heightening the risk of VD deficiency (8).

VD affects various body systems beyond bone health, including the immune system, where it modulates immune responses; the cardiovascular system, which influences heart and blood vessel functions; the endocrine system, particularly insulin secretion and glucose metabolism; and the nervous system, which affects brain function and mental health. These roles highlight the importance of VD in overall health and disease prevention (9).

Therefore, this study aimed to identify factors associated with VD deficiency in Mexican health care workers exposed to SARS-CoV-2. This information may strengthen the importance of vitamin D supplementation in this high-risk group.

Materials and methods

This cross-sectional study was carried out between June 2020 and January 2021 at four tertiary care hospitals treating COVID-19 patients in Mexico City. Participants were enrolled during the period from June 15 to December 15, 2020, encompassing the summer and fall seasons. The inclusion criteria were frontline health care workers who were hospitalized with COVID-19, who underwent confirmatory testing for SARS-CoV-2 due to suspected symptoms or due to having unprotected contact with a suspected case, and who had a blood test for 25-hydroxy vitamin D (25[OH]D) levels. The exclusion criteria included subjects who were already receiving VD or other vitamin supplements and those whose 25[OH]D samples were not processed.

Data from health care workers without COVID-19 were obtained from a previous clinical trial registered at ClinicalTrials.gov (#NCT04535791), and these data were used to analyze the efficacy and safety of VD supplementation in preventing SARS-CoV-2 infection (10).

Participants were divided into two groups: those with VD deficiency (25[OH]D levels <20 ng/mL) and those without (25[OH]D levels ≥20 ng/mL) (11).

As previously described (10), all participants underwent simultaneous serum sampling for 25[OH]D determination and RT–PCR COVID-19 testing, which occurred as part of the RCT selection process. Anthropometric measurements and blood pressure assessments were performed on the participants. Obesity was defined as a body mass index (BMI) >30. Previous diagnoses of type 2 diabetes (T2D) and high blood pressure (HBP) were recorded as comorbidities. HBP was defined as a persistent elevation of blood pressure above systolic pressure ≥ 140 mmHg or diastolic pressure ≥ 90 mmHg (12), and T2D was defined as fasting plasma glucose levels >126 mg/dL or 2-h values in the oral glucose tolerance test of >200 mg/dL (13). A subject was classified as having a positive SARS-CoV-2 infection if they presented a positive real-time polymerase chain reaction (RT–PCR) test.

The protocol was approved by the institutional review boards of the Instituto Mexicano del Seguro Social (CNIS # R-2020-785-090) and Hospital Infantil de Mexico Federico Gómez (HIM-2020-045). All participants provided written informed consent after the procedures were explained according to the protocol.

Laboratory procedures

Vitamin D determination

The 25(OH)D concentrations were determined at the Hospital Infantil de Mexico Clinical Laboratory using the Abbot brand chemiluminescence technique with Architect 1,000 equipment. According to the serum levels, a VD deficiency was defined as <20 ng/mL, VD insufficiency was defined as 20–29.99 ng/mL, and a normal level was defined as >30 ng/mL (11, 14).

Statistical analysis

Using the Kolmogorov–Smirnov test, it was determined that the quantitative variables did not have a Gaussian distribution, so they are presented as median with their respective 95% confidence intervals (95% CIs). Qualitative variables are expressed as proportions and frequencies.

Patients were categorized into two groups: those with and without VD deficiency. VD levels were compared across participants based on their comorbidities and type of personnel. Additionally, serum VD concentrations were compared among factors that could influence levels, such as obesity, T2D, HBP, age, and sex.

To assess differences between groups, we applied the Mann–Whitney U test or Kruskal-Wallis for quantitative variables and the chi–square test for qualitative variables.

Multiple logistic regression analysis was conducted to determine the associations of VD levels with age, male sex, HBP, T2D, and obesity. All the statistical analyses were performed using STATA version 12.0.

Results

Among the 590 potential candidates for inclusion, 122 were excluded: 72 did not meet the inclusion criteria, 18 had samples that were not processed for serum vitamin D levels, and 32 were health care workers who declined to participate. Among the 468 health care workers included, the median age was 40 years, and there was a predominance of female participants (65.4%). Nursing was the most common type of personnel (35.5%), followed by doctors (20.5%) and laboratory personnel (17.7%).

As shown in Table 1, 197 (42.1%) participants had one or more comorbidities, and HBP was the most common ($n = 145$, 31.0%). In total, 151 patients were positive for SARS-CoV-2 (32.3%).

The distribution of VD levels is shown in Figure 1; the median VD level among all health care workers was 16.6 ng/mL (95% CI 17.0–18.2 ng/mL). Among the 24 health care workers with sufficient VD levels, the median age was 39 years (95% CI: 38–46). Of these, 4.2% ($n = 1$) had DM2, 37.5% ($n = 9$) had hypertension, and 20.8% ($n = 5$) were obese.

There was no difference in VD levels according to age or sex, nor by type of health personnel (Figure 2A). However, when comparing between participants with and without VD deficiency (Table 1), a smaller proportion of doctors (18.5% vs. 25.5%) and laboratory workers (15.7% vs. 22.4%) had VD deficiency compared to nurses (36.9% vs. 32.2%), but this difference was not statistically significant. By contrast, participants with T2D and other comorbidities had a statistically higher frequency of VD deficiency ($p < 0.05$), as shown in Table 1.

Serum VD concentrations were also compared among patients according to factors that could influence serum levels. Subjects with

TABLE 1 General characteristics of the health-care workers included.

Participants, No. (%)				
		Vitamin D classification		
Characteristic	Total *n* = 468	<20 ng/mL *n* = 325 (69.4%)	> 20 ng/mL *n* = 143 (30.6%)	*p*-value
Sex				
Female	306 (65.4)	208 (64.0)	98 (68.5)	0.343
Male	162 (34.6)	117 (36.0)	45 (31.5)	
Age, y				
Median (CI 95%)	40 (40–42)	41 (40–43)	39 (38–42)	0.377
Risk factors				
High blood pressure	145 (31.0)	99 (30.5)	46 (32.2)	0.713
Type 2 Diabetes	49 (16.3)	43 (20.8)	6 (6.4)	**0.001**
Obesity	126 (26.9)	96 (29.5)	30 (21.0)	0.054
Comorbidities (≥ 1 of the above)	197 (42.1)	147 (45.2)	50 (35.0)	**0.038**
25 hydroxy- Vitamin D, ng/ml				
Median (CI 95%)	16.6 (17.0–18.2)	14.4 (13.9–14.6)	23.6 (24.3–26.4)	**<0.001**
<20	325 (69.4)	325 (100)	-	**0.001**
20–29.9	119 (25.5)	-	119 (83.2)	
≥ 30	24 (5.1)	-	24 (16.8)	
Type of personnel				0.085
Nurses	166 (35.5)	120 (36.9)	46 (32.2)	
Doctors	96 (20.5)	60 (18.5)	36 (25.2)	
Laboratory workers	83 (17.7)	51 (15.7)	32 (22.4)	
Others	83 (17.7)	63 (19.4)	20 (14.0)	
Orderlies and cleaning staff	40 (8.6)	31 (9.5)	9 (6.3)	
Infection due to SARS-CoV-2				
Positive	151 (32.3)	117 (36.0)	34 (23.8)	**0.009**

BMI, body mass index; CI 95%, confidence interval 95%. BMI is calculated as person's weight in kilograms divided by the square of height in meters. Bold values means statistical significance.

T2D (13.3 ng/mL vs. 17.1 ng/mL, $p < 0.001$), obesity (15.7 ng/mL vs. 17.1 ng/mL, $p = 0.047$), and comorbidities (15.7 ng/mL vs. 17.6 ng/mL, $p < 0.001$) had lower VD levels than those without these factors (Figure 2B). No significant differences were found among participants with HBP (Table 2).

According to the multivariate analysis, T2D (OR 3.40, CI 95%: 1.43 to 8.05, $p = 0.005$) was associated with VD deficiency, adjusted for age, sex, the presence of obesity, and HBP (Table 3).

Discussion

The high prevalence of VD deficiency among Mexican health care workers exposed to SARS-CoV-2 observed in our study is alarming, with 69.4% showing low serum levels. This rate is significantly higher than that seen in the general population but aligns with findings from studies on high-risk occupational groups and individuals with conditions such as obesity and diabetes (2, 15). This rate of VD deficiency, which is higher than the global average of 50%, underscores the severity of the issue. In the Americas, the prevalence of VD deficiency has reached 77% (16). Specifically, in Mexico, the National Health and Nutrition Survey 2006 (ENSANUT) reported a VD deficiency and insufficiency rate of 30% in adults, with Mexico City (area where this study was developed) showing a higher rate than other regions, at 43.1% (17). During the first year of the COVID-19 pandemic, health care workers faced long hours (which reduced sun exposure) and a shortage of medications (including cholecalciferol) and consumed low levels of VD-rich foods, leading to increased rates of VD deficiency. It is crucial to maintain optimal VD concentrations, which, according to the Society of Endocrinology, should be above 30 ng/mL (14, 18). Our findings are similar to those recently reported by Ito et al.; they conducted a study to examine the prevalence of VD deficiency among healthcare workers following the onset of the COVID-19 pandemic (19). They identified that 9.2% of the subjects had adequate VD levels, whereas our data revealed that only 5.1% had adequate VD levels (>30 ng/mL).

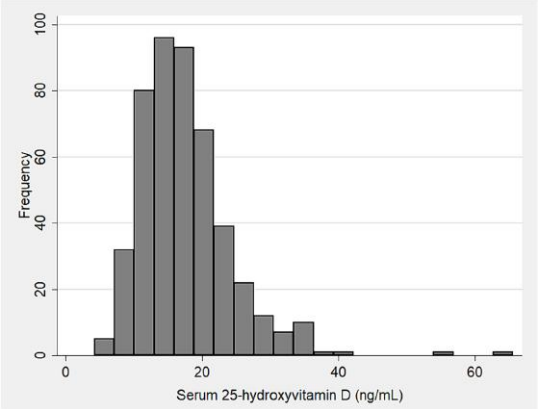

FIGURE 1
Histogram showing the distribution of serum 25-hydroxy vitamin D levels in 468 health-care workers.

Studies focusing on VD deficiency across different occupational and health risk groups have documented varying prevalence rates. For instance, shift workers and indoor workers experience similar trends of VD deficiency due to comparable environmental and work conditions that limit sun exposure, with deficiency rates between 78 and 80% compared to 48% for outdoor workers (2, 20). Recent studies have reported that the prevalence of VD deficiency among health care workers ranges from 45 to 51% (19, 21). These trends, observed across occupations with limited outdoor activity, highlight the occupational risk associated with VD deficiency. These findings emphasize the critical role of occupational factors in VD levels, suggesting that regular screening for VD levels in high-risk occupational groups should be considered in clinical practice guidelines and public health initiatives to prevent adverse health outcomes linked to VD deficiency.

Health care workers often endure prolonged periods indoors with limited exposure to natural sunlight, which is the primary source necessary for synthesizing VD in the skin. This issue is compounded in roles that involve night shifts or extensive indoor duties. The deficiency in VD is due to low UVB exposure and a secondary decrease in vitamin synthesis in the skin with aging (22, 23), as the precursor to VD, 7-dehydrocholesterol, decreases by approximately 50% between the ages of 20 and 80 (23).

Obesity and diabetes merit particular attention among the factors that influence VD levels. The literature presents contradictory results regarding

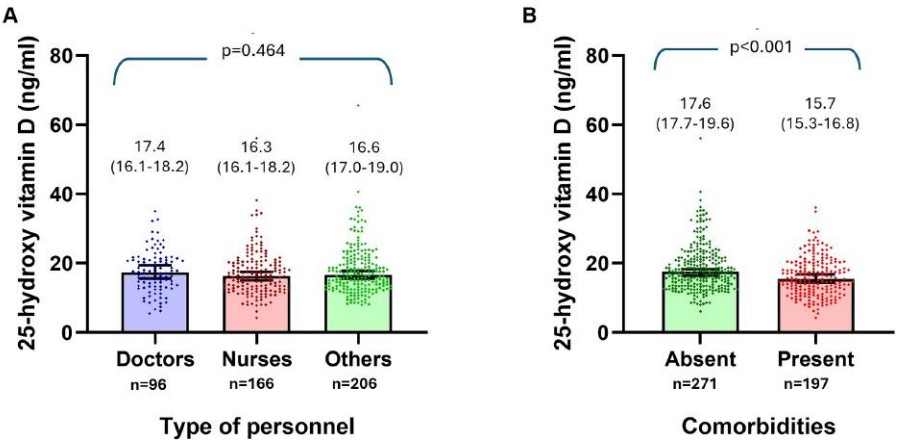

FIGURE 2
Comparison of serum levels of 25-hydroxy vitamin D (ng/ml) according to the type of personnel **(A)**, and by the presence or not of comorbidities **(B)**.

TABLE 2 Comparison of serum levels of 25-hydroxy vitamin D (ng/ml) according to the characteristics of healthcare workers.

	Comorbidities		
	Present	Absent	
Comorbidities	Median (95% confidence interval)		*p*-value
Male sex ($n = 162$)	15.8 (16.4–18.5)	17.0 (16.9–18.4)	0.349
Obesity ($n = 126$)	15.7 (15.5–17.7)	17.1 (17.2–18.7)	**0.047**
Type 2 diabetes ($n = 57$)	13.3 (12.8–15.6)	17.1 (17.4–18.7)	**<0.001**
High blood pressure ($n = 145$)	16.9 (16.5–19.0)	16.5 (16.9–18.2)	0.786
Comorbidities ($n = 197$)	15.7 (15.3–16.8)	17.6 (17.9–19.6)	**<0.001**

Bold values means statistical significance.

TABLE 3 Logistic regression analysis to identify factors related to deficiency vitamin D.

	Adjusted OR	95% confidence interval	*p*-value
Age (years)	0.99	0.97 to 1.01	0.924
Male sex	1.18	0.77 to 1.81	0.438
Type 2 Diabetes	3.40	1.43 to 8.05	**0.005**
Obesity	1.44	0.88 to 2.34	0.141
High blood pressure	0.08	0.52 to 1.25	0.346

Bold values means statistical significance.

the impact of obesity on VD deficiency (24). However, VD deficiency becomes more apparent in obese patients when diabetes mellitus is also present. A study conducted by Atia et al. (25) revealed that the prevalence of VD deficiency was significantly greater in individuals with prediabetes than in those without prediabetes (38.5% vs. 25.5%). In obese individuals, VD tends to be sequestered within adipose tissue, reducing its availability in the bloodstream. This biological trapping of VD in fat cells diminishes its overall active circulation (26). In the context of diabetes, the disease's impact on renal function and liver enzymes can interfere with the hydroxylation processes that are essential for the vitamin's activation. High glucose levels, which are common in diabetes, may further impair these processes, complicating the metabolic pathway required for converting VD to its active form (27, 28). These interconnected mechanisms highlight a compounded risk where occupational and personal health factors converge, leading to increased vulnerability to VD deficiency.

Study limitations include the inability to specify the work schedules of the included workers, as shifts varied during the pandemic. The study did not analyze the seasons during which participants were included; however, only non-winter seasons, namely, summer and autumn, were considered; winter, which is associated with decreased serum VD levels, was not included. The major limitation was the cross-sectional study design, which could not establish a causal relationship between VD deficiency and modifying factors such as obesity and diabetes.

Given the high prevalence of VD deficiency among health care workers, particularly those with prolonged indoor exposure and minimal sunlight, it is imperative to routinely evaluate serum VD levels. For health care workers, especially those positive for SARS-CoV-2, the benefits of VD supplementation could extend beyond correcting the deficiency—it might also enhance immune response capabilities against viral infections. Considering the broader implications, maintaining adequate vitamin D levels is crucial for infectious disease prevention and general health maintenance within health care settings.

In conclusion, the frequency of low VD values is high among health care workers, and TD2 was a significant risk factor associated with these values. This study underscores the urgent need for systematic VD deficiency screening and tailored VD supplementation strategies. Such measures are crucial not only for the health of individual workers but also for supporting the operational integrity and resilience of health care systems facing ongoing public health challenges.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by The protocol was approved by the institutional review boards of the Instituto Mexicano del Seguro Social (CNIS # R-2020-785-090) and Hospital Infantil de Mexico Federico Gómez (HIM-2020-045). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MV-K: Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing. JZ-C: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. JG-E: Funding acquisition, Supervision, Writing – review & editing. ML-A: Resources, Supervision, Writing – review & editing. AB: Data curation, Investigation, Writing – review & editing. MM-N: Formal analysis, Investigation, Writing – review & editing. IP-O: Formal analysis, Investigation, Writing – review & editing. BL-M: Investigation, Writing – review & editing. HG: Writing – review & editing, Investigation. MK-K: Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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No causal relationship between serum vitamin D levels and alcoholic liver disease: a two-sample bidirectional Mendelian randomization study

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Background: Numerous observational studies have presented an association between Vitamin D (VD) and Alcoholic Liver Disease (ALD). However, sufficient evidence from Randomized Controlled Trials (RCTs) substantiating this correlation is scarce, thus leaving the causality of this relationship ambiguous. To overcome the shortcomings of traditional observational studies, we performed a two-sample bidirectional Mendelian randomization (MR) analysis to ascertain the causal relationship between VD and ALD.

Methods: We utilized summary statistics datasets from Genome-Wide Association Studies (GWAS) for VD and ALD. We selected genetic instruments that measure circulating VD levels (n = 64,979), and retrieved ALD statistics from GWASs, inclusive of 1,416 cases and 217,376 healthy controls, while excluding chronic liver diseases such as nonalcoholic fatty liver disease, toxic liver disease, and viral hepatitis. Subsequent, MR analyses were performed to obtain effect estimates using inverse variance weighted (IVW) random effect models. Cochran's Q statistic and MR-Egger regression intercept analyses were used to assess pleiotropy. Sensitivity analyses using the MR Egger, weighted median, simple mode, and weighted mode methods were also performed. Leave-one-out analysis was used to identify SNPs with potential effect. Reverse MR analysis was also performed.

Results: In IVW, our MR analysis incorporated 21 independent SNPs, circulating VD levels had no causal effect on ALD [OR = 0.624 (0.336–1.160), p = 0.136] and ALD had no causal effect on circulating VD [OR = 0.997 (0.986–1.008), p = 0.555]. No heterogeneity or pleiotropy was observed (p > 0.05). Other MR methods also agreed with IVW results.

Conclusion: This study provides the causal relationship between genetically predicted circulating Vitamin D levels and ALD and provides new insights into the genetics of ALD.

KEYWORDS

vitamin D, alcoholic liver disease, GWAS, Mendelian randomization, SNPS

Introduction

Alcoholic Liver Disease (ALD) stands out as a significant global contributor to liver-related disorders, stemming from the detrimental impact of prolonged and excessive alcohol consumption. Notably, recent years have witnessed a consistent rise in the prevalence of alcohol consumption and alcohol addiction on a global scale. This surge has consequently been linked to an escalated overall mortality rate, thus further accentuating the substantial clinical and socioeconomic burden associated with ALD. Nevertheless, within this intricate landscape, the interplay between ALD, genetic susceptibility, and environmental influences remains multifaceted. The deficiency of key dietary nutrients, VD being a pertinent example, has been identified as a potential factor in the initiation of ALD (1, 2).

Vitamin D is produced by the skin upon exposure to sunlight and are also obtained through the diet, exhibits diverse physiological functions. Post synthesis or ingestion, these hormones undergo hydroxylation in the liver to form 25-hydroxyvitamin D [25(OH)D], which is the main circulating form of VD3 in humans. VD not only affects bone and calcium metabolism function, but also reduces the risk of chronic diseases, which including diabetes, cancer, cardiovascular, infectious, and auto-immune diseases (3). In recent years, in the mechanisms of liver inflammation and injury induced by ethanol, the dysfunction of anti-inflammatory and antioxidant functions may exacerbate the progression of the disease. The anti-inflammatory and antioxidant effects of VD have become a focal point of research, particularly concerning its role in the occurrence and development of chronic liver diseases (4, 5). Accumulated observational studies in humans show an inverse relationship between VD levels, and the risk and severity of ALD (2, 4, 6). Despite several studies reporting decreased VD levels in ALD patients and suggesting potential benefits of enhancing VD levels, the effect of VD supplementation in these patients remains debatable (7). Establishing causality remains challenging due to potential confounders and reverse causation.

Mendelian randomization is an epidemiological tool used for establishing causal inference between exposure and outcomes by employing genetic variation as instrumental variables (IVs). This allows MR to overcome the limitations of traditional observational studies and significantly eliminating reverse causation (8). Nonetheless, to date, there have been no MR studies to investigate the causal relationship between serum VD levels and ALD.

The definition of outcome and selection of instrumental variables critically influence MR findings. This study aimed to assess the risk of ALD in the United Kingdom Biobank (UKBB) cohort by conducting GWAS with a broader case definition than employed in previous works. Subsequently, utilizing genetic instruments derived from a European population's meta-analysis GWAS on VD status, we conducted a two-sample bidirectional MR analysis to estimate first the effect of genetically predicted serum VD levels on risk of ALD, and reciprocally to estimate the causal effect of genetic risk for ALD on serum VD levels.

Abbreviations: ALD, Alcoholic liver disease; GWAS, Genome-wide association studies; SNPs, Single nucleotide polymorphisms; MR, Mendelian randomization; IVs, Instrumental variables; IVW, Inverse variance weighted; VD, Vitamin D; 25(OH)D, 25-hydroxyvitamin D.

Materials and methods

The design of this two-sample bidirectional MR is summarized conclusion, which illustrated in Figure 1.

Software selection and data source

We conducted our search on the MR Base database,[1] a repository containing a substantial number of summary statistic data from hundreds of GWASs (9). To mitigate any potential bias that might arise due to population stratification, we only included subjects of European genetic origin in our study. The summary statistics datasets for VD, publicly available and derived from GWAS meta-analyses concerning European individuals ($n = 64{,}979$; GWAS ID: ukb-b-18593), served as our exposure. We sourced the ALD dataset from the most extensive histology-based ALD GWAS, comprising 1,416 European ALD cases and 217,376 genetically matched controls (GWAS ID: finn-b-ALCOLIVER). Each dataset was obtained from the published summarized results of publicly available, genome-wide association studies.

Selection of the genetic instrument

The effect of VD levels on the risk of ALD (Variable 1, Figure 1) was evaluated using SNPs discovered in the Study of Underlying Genetic Determinants of VD, which showed an association with VD status. These SNPs were utilized as IVs to examine the correlation between genetically-inferred serum VD levels and ALD risk in the UKBB population cohort. A two-sample MR study of genetic variants linked with VD was employed as the IV, refining inference based on a p value threshold of $1^{*}10^{-5}$ to capture as many potential genetic variants as possible. We procured summary statistics, including beta coefficients and standard errors, for 21 SNPs associated with VD, using these as IVs based on data from GWASs on VD. Conversely, we investigated the effect of ALD risk on VD levels using data from GWAS on ALD, which identified 21 standalone, genome-wide significant SNPs (Variable 2, Figure 1).

Statistical analysis for Mendelian randomization

Mendelian randomization analysis is a statistical technique that requires genetic variants to be related to the exposure of interest, but not potential confounders, to establish causal relationships (10). Our study followed a three-step approach to investigate the association between VD and the risk of ALD. Firstly, we examined the independent association of SNPs with VD levels and the risk of ALD. Secondly, we assessed the association between each SNP and the risk of ALD. At the same time, we assessed the association between each SNP and VD levels. Lastly, we utilized two-sample bidirectional MR analysis, a method that leverages summary statistics from

1 http://www.mrbase.org/

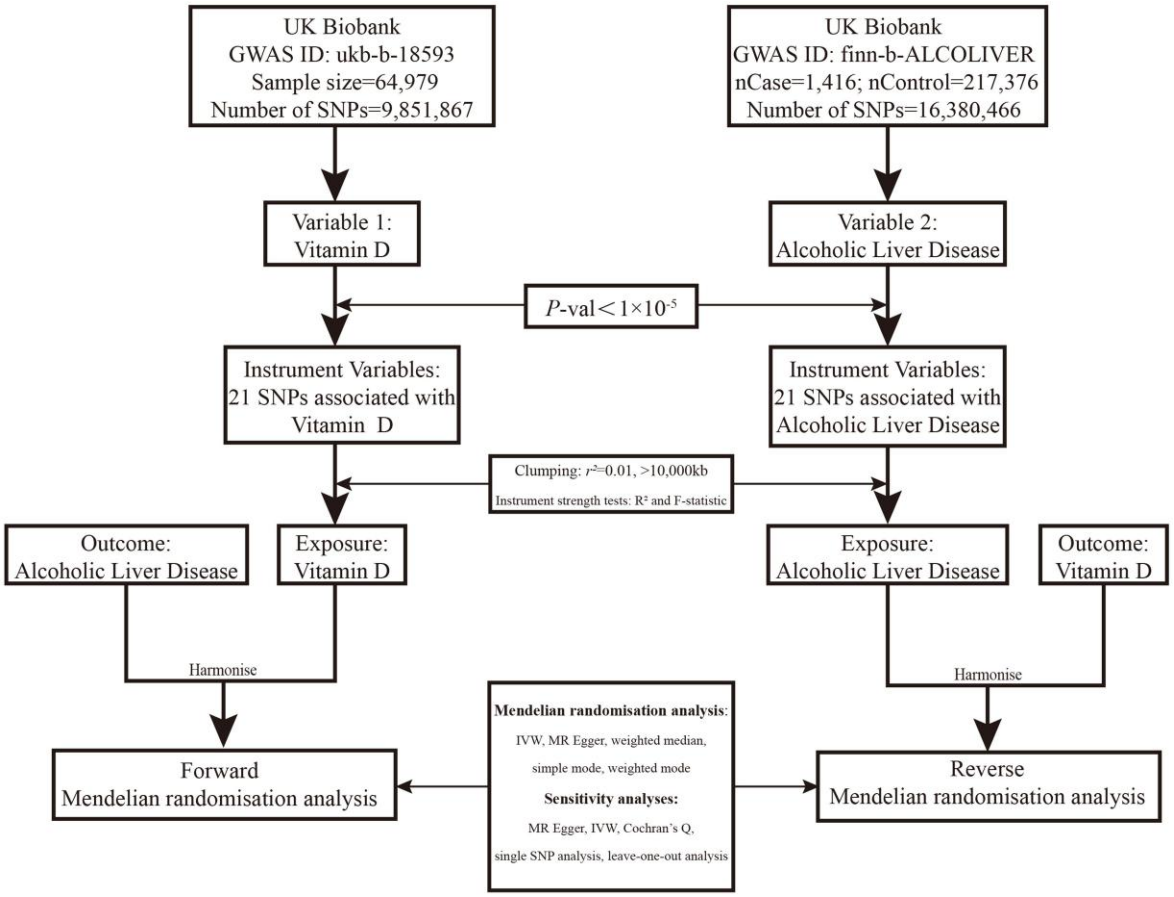

FIGURE 1
Overview of the two-sample MR study design used to investigate the probability of bidirectional association between serum VD and ALD. VD, Vitamin D; GWAS, Genome-wide association study; IVW, Inverse-variance weighted; MR, Mendelian randomization; ALD, Alcoholic liver disease; and SNP, Single-nucleotide polymorphism. SNPs were included if identified to be associated with ALD in the Speliotes' GWAS with effects in the same direction with a *p* value of $<1*10^{-5}$ in UKBB or were "discovered" in our GWAS in UKBB with a *p* value of $<1*10^{-5}$.

different GWASs (11), to estimate the causal relationship between VD and the risk of ALD. For this analysis, we employed 21 SNPs as IVs obtained from the VD and ALD GWASs. By using this MR approach, we aimed to derive an unbiased estimate of the causal association between VD and the risk of ALD, while minimizing the influence of confounding factors (Table 1).

In this study, the IVW method amalgamates the Wald ratio estimates of the causal effect procured from multiple genetic variants, lending a consistent estimation of the causal effect of the exposure variable on the outcome (12). To tackle potential pleiotropy, where genetic variants might influence multiple variables, we incorporated two additional methods: MR-Egger regression and the weighted median estimator. MR-Egger regression addresses unbalanced pleiotropy, factoring in a parameter for bias, via the use of summary data estimates of causal effects from each genetic variant (13). It executes a weighted linear regression of gene-outcome coefficients on gene-exposure coefficients, wherein the slope embodies the causal effect estimate. The average horizontal pleiotropic effect across genetic variants is estimated by the intercept (14). The weighted median estimator, on the other hand, provides a consistent estimate of the causal effect even if up to 50% of the information comes from genetic variants that are not valid instrumental variables (15). Sensitivity analyses using the MR Egger, weighted median, simple mode, and weighted mode methods were also performed (13, 15, 16). As each method makes slightly different assumptions, a consistent effect across multiple methods yields the most robust evidence of causal inference.

TABLE 1 The results of heterogeneity and sensitivity test.

	Methods	Q	df	Q-val	I^2
Heterogeneity test	Vitamin D on ALD				
	MR Egger	19.076	19	0.452	0.004
	Inverse variance weighted	19.327	20	0.501	0.035
	ALD on Vitamin D				
	MR Egger	16.054	19	0.654	0.184
	Inverse variance weighted	17.670	20	0.610	0.132
Sensitivity test	Egger regression intercept	Standard error		Directionality *p* value	
	Vitamin D on ALD				
	0.016	0.032		0.623	
	ALD on Vitamin D				
	0.006	0.005		0.219	

A higher R^2 and F-statistic denote a reduced risk of weak instrument bias, and hold greater precision in estimations compared to MR-Egger analyses. Statistical significance was set to $p < 0.05$. We ran all Mendelian randomization analyses on RStudio Software (Version: 2023.06.0 Build 421) and *R* Software (Version: 4.3.1) (Supplementary material 1).

Heterogeneity and sensitivity test

We assessed the heterogeneities between SNPs using Cochran's *Q*-statistics (17) band I^2 statistic (18, 19). Additionally, we also conducted a "leave-one-out" analysis to explore the possibility of a causal association driven by a single SNP.

Results

Studies included in the meta-analysis

Instrumental variables for Mendelian randomization

In our analysis, we utilized a set of 21 independent SNPs identified from GWASs of VD and ALD as IVs. Each of these SNPs demonstrated a significant association with VD and ALD as per the genome-wide level of significance (refer to Supplementary Table S3 for more details). Of note, the *F* statistic, denoting the robustness of the IVs, was recorded to be 10 or above for each individual SNP variant. Conventionally, an *F* statistic under 10 typically signifies a "weak IV," indicating that our study had minimal risk of weak instrument bias. The *F* statistic for all the SNPs used in the MR analysis was >10, verifying them as "strong" instruments. The *F* statistic measures the magnitude and precision of each SNP's influence over VD and ALD. The individual *F* statistic for VD ranged between 20 and 23 (refer Supplementary Table S1) and for ALD, it ranged between 20 and 85 (refer Supplementary Table S2).

Mendelian randomization results

The IVW random method revealed no significant effect of serum VD levels on the risk of ALD, with an OR of 0.624 (0.336, 1.160) and a p value of 0.136 (see Table 2 and Figures 2, 3). Conversely, the IVW random effect analysis also found no evidence of a causal effect of ALD on the odds of VD, with an OR of 0.997 (0.986, 1.008) and a p value of 0.555 (consult Table 2 and Figures 2, 3). The intercept of the MR-Egger test, representing the mean pleiotropic effect across genetic variants, was insignificantly different from zero, suggesting that directional pleiotropy is unlikely to bias the results (refer to Table 1). Furthermore, an evaluation involving the MR-Egger analysis, weighted median, weighted mode, and simple mode found no causal association between VD and ALD.

Heterogeneity and sensitivity test

The Cochran's *Q* test was conducted to evaluate heterogeneity among instrumental variable estimates from individual genetic variants. The results did not indicate any substantial evidence of heterogeneity (refer to Table 1 and Figure 4). Heterogeneity is the variability in causal estimates obtained from each SNP. A low heterogeneity value signifies an increase in the reliability of MR estimates. Further strengthening the reliability of MR estimates, the I^2 values also displayed low heterogeneity (refer to Table 1). In the "leave-one-out" analysis, each SNP was separately excluded to analyze its effect on the overarching IVW point estimate (refer to Figure 5). It was revealed that no single SNP had a significant impact on the IVW point estimate. This suggests that the cumulative result was not skewed by any particular genetic variant. A funnel plot was used to evaluate publication bias and directional horizontal pleiotropy, and it did not display any significant asymmetry. Furthermore, the MR-Egger regression test did not show any evidence of asymmetry, thereby further asserting the absence of bias due to directional horizontal pleiotropy (refer to Figure 4). Conclusively, the lack of considerable heterogeneity, low I^2 values, outcomes of the "leave-one-out" analysis, and absence of asymmetry in the funnel plot and MR-Egger regression test affirm the reliability of the MR estimates and reduce apprehensions about potential analysis biases.

TABLE 2 Results of two-sample bidirectional MR analysis of the causal effects between Vitamin D and ALD.

Exposures	Outcomes	Methods	Number of SNPs	Beta	SE	*p* value	OR	95%CI
Vitamin D on ALD								
Vitamin D	ALD	MR Egger	21	−0.839	0.802	0.308	0.432	0.090–2.081
		Weighted median	21	−0.046	0.466	0.921	0.955	0.383–2.381
		Inverse variance weighted	21	−0.471	0.316	0.136	0.624	0.336–1.160
		Simple mode	21	0.274	0.888	0.761	1.315	0.231–7.502
		Weighted mode	21	0.293	0.876	0.741	1.341	0.241–7.465
ALD on Vitamin D								
ALD	Vitamin D	MR Egger	21	−0.016	0.012	0.178	0.984	0.962–1.007
		Weighted median	21	0.000	0.008	0.955	1.000	0.984–1.017
		Inverse variance weighted	21	−0.003	0.006	0.555	0.997	0.986–1.008
		Simple mode	21	0.004	0.015	0.774	1.004	0.976–1.034
		Weighted mode	21	0.007	0.016	0.656	1.007	0.976–1.040

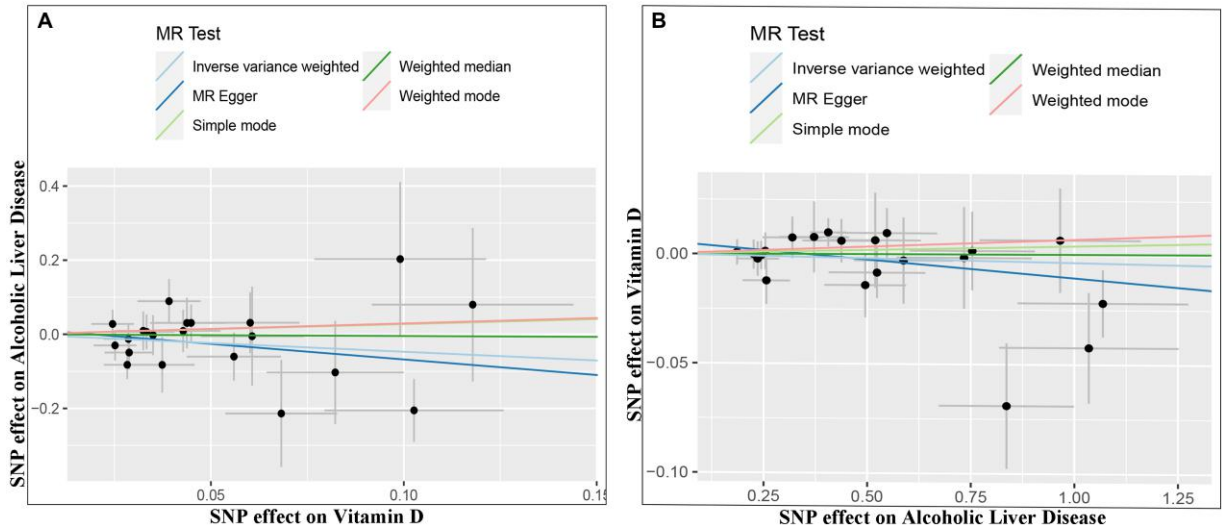

FIGURE 2
Scatter plots of genetic associations between Vitamin D and ALD. The slopes of each line represent the causal association for each method. The light blue line represents the inverse-variance weighted estimate, the green line represents the weighted median estimate, the dark blue line represents the Mendelian randomization-Egger estimate, the red line represents the weighted mode estimate, and the light green line represents the simple mode estimate (**A**, Effect of Vitamin D on ALD; **B**, Effect of ALD on Vitamin D).

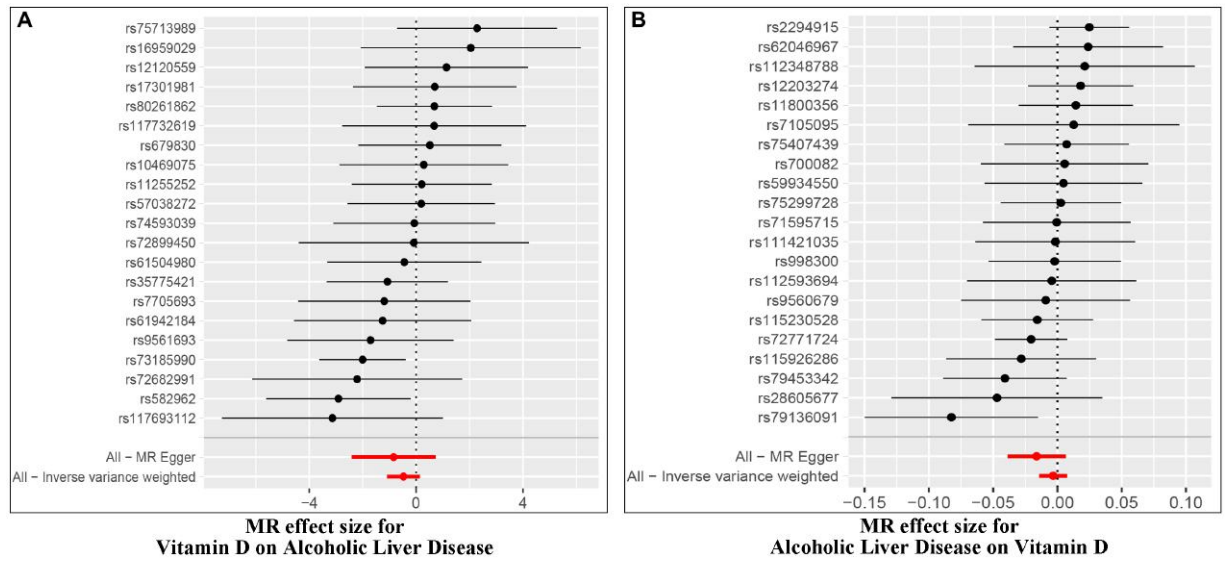

FIGURE 3
Forest plot of MR effect of the causal relationship between Vitamin D and ALD (**A**, Effect of Vitamin D on ALD; **B**, Effect of ALD on Vitamin D).

Discussion

Existing studies have noted a link between serum VD levels and ALD (1, 2, 6). However, it is still uncertain whether this association is causal or the direction of the causality. In this extensive two-sample bidirectional MR study exploring the ViD and ALD relationship, we found no tangible evidence supporting a reciprocal causal link between serum VD levels and ALD susceptibility in a large cohort of European ancestry. To the best of our knowledge, this is the inaugural two-sample MR examination inspecting the link between serum VD levels and the risk of ALD within a European population.

Vitamin D deficiency is a widespread condition that has reached epidemic proportions in Western countries (20), primarily due to current lifestyle and limited dietary sources. An estimated billion people are deficient in VD (21). With anti-inflammatory, immunomodulatory, proapoptotic, and antiangiogenic effects, VD operates critical roles within the body (22–24), including protection against rickets/bone demineralization, hypertension, tumor, the body's defense against infections, and autoimmune (25, 26). A damaging effect of VD insufficiency on the immune system could happen during severe chronic liver diseases (3). Besides, VD is involved in regulating adipose tissue inflammation, liver fibrosis, and

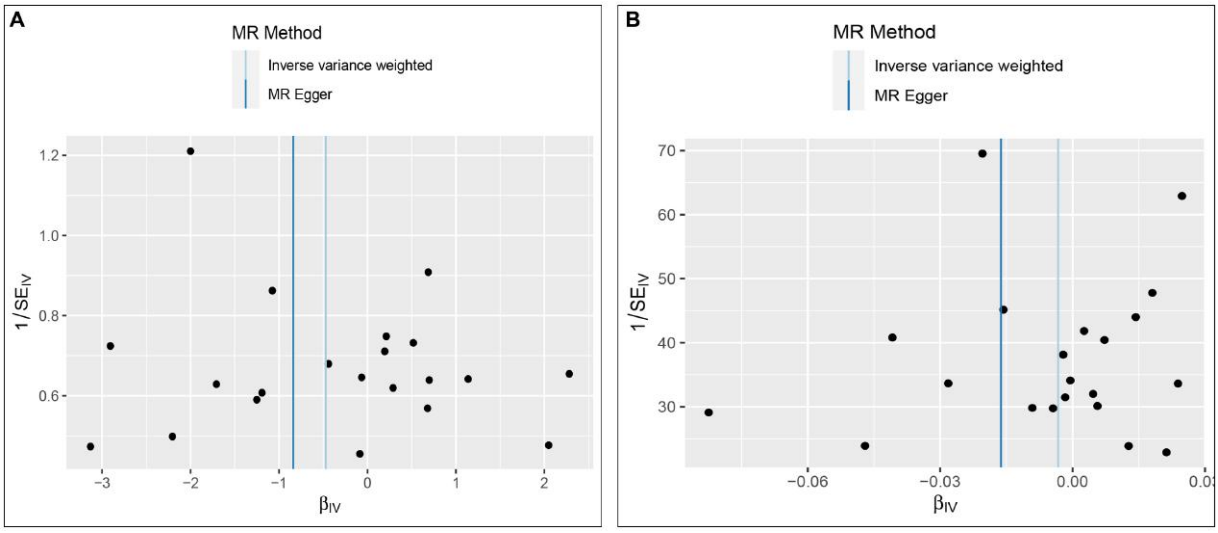

FIGURE 4
Funnel plot to assess heterogeneity. The light blue line represents the inverse-variance weighted estimate, and the dark blue line represents the Mendelian randomization-Egger estimate (**A**, Effect of Vitamin D on ALD; **B**, Effect of ALD on Vitamin D).

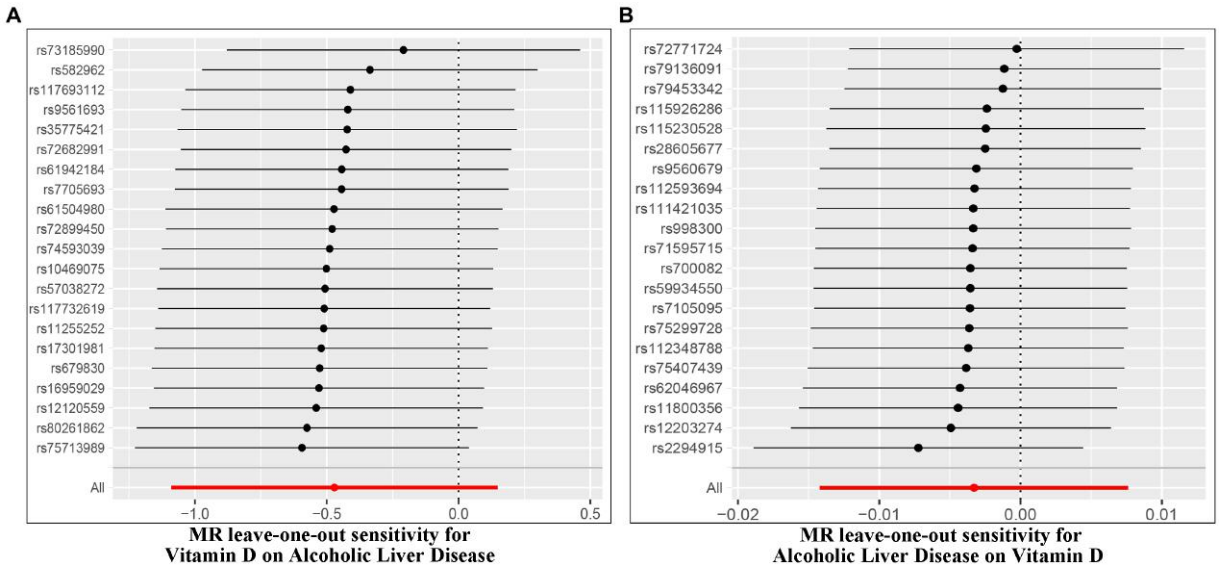

FIGURE 5
Leave-one-out of SNPs associated with Vitamin D and ALD. Each black point represents result of the IVW MR method applied to estimate the causal effect between Vitamin D and ALD [**(A)** Effect of Vitamin D on ALD; **(B)** Effect of ALD on Vitamin D].

predicting antiviral effects (27–30). It also influences insulin resistance and abnormal fat accumulation in the liver (24, 31, 32). Recently, reports have indicated VD deficiency in chronic liver diseases regardless of the etiology (33).

There is significant scientific interest in the connection between VD status and ALD. Observational studies have observed decreased VD levels in ALD (34). VD deficiency is a contributing factor to ALD (6). For instance, VD was an independent cofactor linked with the occurrence of ASH in alcoholic patients, who frequently had severe VD deficiency and bridging fibrosis (2). Its mode of action might be connected to the activation of the NF-κB signaling pathway, which is associated with promoting the inflammatory response (35). A relationship between severe VD deficiency exists and the mortality rate in alcoholic cirrhotic patients (4, 31). Consequently, VD has been regarded as a risk aspect for the evolution of ALD. Furthermore, SNPs within the VD receptor (VDR) gene have a recognized association with chronic liver disease (36, 37). In a study conducted by Saberi et al. (38), they proposed that the VDR activation signal interferes with the transforming growth factor beta-dependent transcriptional responses in profibrotic genes in HSCs. The VDR agonist calcifertriol reduces liver fibrosis in a mouse model of liver injury. Yet, as several ALD patients do not exhibit low VD levels, questioning the causality of VD ensues. While a large body of preclinical and observational data proposes a relationship between VD status and risk and severity of

ALD (6), solid evidence from clinical intervention trials remains insufficient (39). A Cochrane review focusing on chronic liver disease in adults concluded that the current evidence does not support the use of VD supplements for the prevention or treatment of these conditions (7). Thus, it remains unclear whether VD has a causal association with ALD.

The MR methodology uses genetic variants linked with a modifiable exposure or biological intermediate to estimate the causal relationship between these variables and a medically pertinent outcome (40). MR circumvents many constraints of conventional epidemiological studies. The random distribution of genetic variants at conception minimizes confounding from environmental factors, thereby fortifying causal inference (41). MR analyses lessen confounding and reverse causality due to the parental random allocation of genotypes to offspring (42). To date, our current MR study is the first two-sample bidirectional MR evaluation to assess the causal role of VD for ALD in the European population. It merits mentioning that total serum VD incorporates VD bound to the VD binding protein (approximately 85%), VD bound to albumin (about 15%), and the fraction of free VD (less than 1%) (43). Contemporary studies have suggested that free or active serum VD may be a superior indicator of VD status compared with total serum VD. This is especially applicable in conditions like pregnancy, liver disease, or kidney disease, which influence VD binding protein levels (43, 44). Therefore, free VD was used as the phenotype in this study, and the genetic tools selected covered genetic variants associated with free serum VD. However, it is worth noting that general and clinical population-level correlations between free and total serum VD have mitigated this limitation to some extent (45). In this study, we executed five different estimation methods (inverse-variance weighted method, weighted median method, weighted mode, simple mode, and MR-Egger regression) for MR analyses. We applied the two-sample MR to assess the association between VD and ALD in this study. The findings indicated a lack of genetic evidence to conclusively support a causal relationship between VD levels and the risk of ALD. This conclusion remained unwavering even in the wake of sensitivity analyses and further replication. In addition, we further divided VD levels into high or low groups and conducted a Mendelian randomization study on the relationship between dichotomous VD levels and ALD risk. Since we did not find a GWAS dataset to obtain high VD levels as an exposure factor, and VD deficiency is more common in clinical practice. Therefore, we conducted a supplementary study focusing on the association between VD deficiency and ALD risk. Our results are consistent with the current study, indicating that there is no causal relationship between VD deficiency and increased ALD risk (see Supplementary material). This may provide additional perspectives on the relationship between VD levels and ALD risk.

Divergences between conclusions may be related to several reasons. On one hand, we tend to suggest that this discrepancy could hint at the flaw (residual confounding) of cross-sectional studies. The remaining association will often still be a biased estimate due to the existence of unknown or unmeasured confounders [sun exposure, physical activity, obesity, insulin resistance, different sample sizes, races, Body Mass Index (BMI), and so on], or imprecision in measured confounders (31). On the other hand, observational studies can be hindered by confounding or reverse causation (46). In addition, given the multifaceted roles of VD in the body, its causal relationship with ALD may be influenced by a variety of physiological and environmental factors. This could potentially explain our observed lack of significant results.

Strengths and limitations

This research encompassed several strengths and limitations. Firstly, the exact function of certain SNPs remains unknown, potentially allowing residual bias when examining pleiotropy. However, we procured consistent results utilizing five MR methods considering pleiotropy, robust methodology, and sensitivity analyses that excluded SNPs with pleiotropic impacts, which is reassuring. Despite the fact that the SNPs used as instruments in our MR were extracted from GWAS in Europeans, the populations of both GWAS were not homogenous in terms of geographic location. It has been shown that there could be some gene–environment interaction in the effect of SNPs in the VD receptor gene on Chronic liver disease risk (36, 37). This raises a possibility of gene–environment interaction for SNPs affecting VD levels and of nonlinear effects of these SNPs on risk of ALD, but two-sample MR studies can only assess linear associations. Secondly, the study population consisted of Europeans, and there were differences in sample size between the VD and ALD datasets. Therefore, Our MR results cannot be generalized to non-Europeans and potentially to Europeans residing in different geographic areas than those of the participants in the VD and ALD GWAS (47). Due to ethnic differences in exposure and outcome GWAS populations, we also cannot completely rule out residual confounding by population stratification (46). We believe that potential biases can be avoided in the future by including more databases of people of non-European ancestry and increasing the sample size. Thirdly, owing to the unavailability of specifics about participant overlap between the two published GWAS summary datasets, it was not possible to compute potential biases arising from participant overlap. Last but not least, the use of two-sample Mendelian randomization enabled us to conduct the largest genome-wide association study on ALD yet undertaken, improving the likelihood of establishing a causal relationship between VD levels and ALD risk. There was less likelihood of confounding and reverse causality bias in this study than in previous routine observational studies.

The future course of this research encompasses broadening the MR strategy to populations beyond European descent. The work will investigate the potential alteration of genetically forecasted VD influences on ALD risk and severity subject to ALD risk factors. These potential aspects include race, ethnicity, age, sex, BMI, and prospective MR analyses, employing updated GWAS samples along with different cohorts.

Conclusion

In conclusion, despite cross-sectional studies exhibiting an association between Vitamin D concentration and ALD in both mappings. Our study suggested that no causal relationship was found between Vitamin D deficiency and ALD; neither did Vitamin D deficiency pose a risk factor for the development of the disease.

Negative results are not meaningless, and many current MR studies have broken the conclusions of observational studies (48, 49). In the future, there is a need for a larger sample size and GWAS data of non-European ancestry patients to update the conclusion.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://gwas.mrcieu.ac.uk/.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

HW: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Writing – review & editing. LW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review & editing. QZ: Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. CL: Conceptualization, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. H-yL: Conceptualization, Funding acquisition, Writing – review & editing. B-fZ: Conceptualization, Funding acquisition, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1292954/full#supplementary-material

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Changes in vitamin D status among adults from the COVID-19 pandemic to post-pandemic normality

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Introduction: The COVID-19 pandemic has prompted widespread lockdown measures globally, significantly impacting daily activities and outdoor exposure. This study investigates the effect of the pandemic on vitamin D levels and the prevalence of vitamin D deficiency in the adult population, with a focus on gender-specific differences.

Methods: A total of 1525 adults from Henan Provincial People's Hospital were included. Serum 25-hydroxyvitamin D (25(OH)D) levels were measured using the Elecsys® Vitamin D total kit on the Roche Cobas® 8000 analyzer. The Clinical Application Consensus on Vitamin D and Its Analogs defined deficiency as 25(OH)D levels below 20 ng/ml. Statistical analysis was performed using SPSS 23.0 and GraphPad Prism 8 software.

Results: The overall 25(OH)D levels increased from 18.14 ng/ml [IQR: 13.78, 23.68] in 2022 to 19.15 ng/ml [IQR: 14.88, 25.01] in 2023 (p=0.004). Males exhibited significant improvement in 25(OH)D levels from 18.01 ng/ml [IQR: 14.10, 23.53] in 2022 to 20.49 ng/ml [IQR: 16.11, 26.01] in 2023 (p<0.001). The prevalence of vitamin D deficiency decreased from 62% in 2022 to 54.9% in 2023 (p=0.009), with a notable reduction in males (64.1% in 2022 to 47.2% in 2023). Among 168 individuals tested in both years, 25(OH)D levels increased from 20.73 ± 9.37 ng/ml in 2022 to 22.28 ± 8.59 ng/ml in 2023 (p=0.012), and the deficiency rate decreased from 58.3% in 2022 to 47.0% in 2023 (p=0.038). The 40–49 age group showed significant improvement in 25(OH)D levels from 16.10 ng/ml [IQR: 12.41, 21.18] in 2022 to 18.28 ng/ml [IQR: 13.91, 23.86] in 2023 (p=0.005), with a reduction in deficiency rate from 72.8% to 59.9% (p=0.02). Furthermore, in February, March, and April, 2022, 25(OH)D levels were significantly lower compared to 2023 (p<0.001, p=0.002, p<0.001, respectively), accompanied by a higher prevalence of vitamin D deficiency (p<0.001, p=0.015, p<0.001, respectively).

Discussion: This study demonstrates that the COVID-19 pandemic significantly impacted vitamin D levels, leading to an increased prevalence of deficiency, particularly among males. These findings highlight the critical importance of maintaining sufficient outdoor activities to ensure adequate vitamin D levels. The data underscore the need for public health strategies to address potential deficiencies during prolonged periods of limited outdoor exposure.

KEYWORDS

vitamin D, adults, COVID-19, pandemic, China

1 Introduction

Since the emergence of the COVID-19 pandemic in Wuhan, China, in early 2020, China has implemented stringent measures to control the spread of the virus (1). These measures entail mandatory mask usage, strict enforcement of social distancing, and stay-at-home orders when necessary. While some regions in China adopted a "zero-COVID" policy starting from August 2021, the city of Zhengzhou in Henan Province enforced rigorous lockdown measures during specific periods: January 5 to February 9, May 3 to May 17, and October 14 to December 14, 2022. These measures significantly curtailed outdoor activities, potentially leading to diminished levels of vitamin D, which is primarily synthesized in the skin through exposure to sunlight, specifically ultraviolet B radiation (2).

Vitamin D serves as a prohormone for a lipid-soluble steroid hormone. In its native form, vitamin D does not exhibit biological activity and necessitates hepatic conversion to its principal storage form, 25(OH)D (3). Following this, it undergoes intracellular transformation within vitamin D receptor-expressing cells, culminating in the generation of the biologically active metabolite, 1,25-dihydroxyvitamin D (4). Vitamin D plays a critical role in mediating calcium absorption and modulating bone metabolism within the human body (5). Moreover, vitamin D deficiency has been linked to unfavorable outcomes in COVID-19 patients, as well as increased susceptibility to bacterial and viral infections (6–8). Some studies have identified a strong correlation between low vitamin D levels and worse COVID-19 outcomes, independent of initial disease severity. This excludes reverse causality and supports vitamin D as an independent predictor of disease progression (9). Furthermore, emerging data have provided intriguing insights into the association between vitamin D levels and Long COVID occurrence. Long COVID, characterized by prolonged symptoms following the acute phase of infection, has been associated with various risk factors, including low vitamin D levels (10). This expands the potential therapeutic applications of vitamin D in managing COVID-19 and its associated comorbidities.

Vitamin D exerts influence on the expression of over 1,000 genes and is implicated in various conditions, including diabetes, diverse cancer types, cardiovascular diseases, autoimmune disorders, and congenital immune disorders (6, 11–13). There is also growing evidence linking vitamin D levels with the response to anti-SARS-CoV-2 vaccination (14). Vitamin D, known for its immunomodulatory effects, may influence the efficacy of vaccines, making it a critical factor to consider in vaccination strategies. Moreover, the non-skeletal effects of vitamin D have been the subject of recent international consensus, which emphasizes its role beyond bone health (15). Of particular interest is the association between vitamin D and diabetes, where recent findings suggest a beneficial effect of adequate vitamin D levels in reducing the risk of diabetes (16).

Previous studies have yielded conflicting findings regarding the impact of the COVID-19 pandemic on vitamin D levels and deficiency rates. Some studies have reported no effect or even an elevation in vitamin D levels and a reduction in deficiency rates among populations (17–19). Conversely, other studies have observed a decline in vitamin D levels and an increase in deficiency rates (20). Currently, there is a dearth of research specifically investigating the influence of the COVID-19 pandemic on vitamin D levels in the adult population of China. Therefore, the primary objective of this study is to perform a comprehensive statistical analysis and comparative assessment of vitamin D levels among a population of healthy adults residing in Zhengzhou, Henan Province. Specifically, we aim to investigate potential variations in vitamin D levels and the prevalence of vitamin D deficiency between two distinct time periods: the COVID-19 pandemic in 2022 and the subsequent post-pandemic phase in 2023. The overarching aim is to elucidate the potential impact of the COVID-19 epidemic on vitamin D levels and the associated deficiency rates.

2 Methods

2.1 Study population

A cohort of 1,525 individuals who underwent comprehensive health examinations at the Henan Provincial People's Hospital Examination Center between January 2022 and December 2023 was selected as the study population. The cohorts in 2022 and 2023 are distinct groups, with 2022 representing data during the pandemic and 2023 representing post-pandemic data. The cohorts consisted of 776 males and 749 females. The inclusion criteria for the study population were as follows: (1) age 18 years or older, and (2) absence of known medical conditions associated with abnormal levels of 25(OH)D, such as hyperparathyroidism or hypoparathyroidism, hyperthyroidism, severe liver or kidney diseases, post-gastrectomy status, severe infection, malignant tumors, among others. The study protocol adhered to the principles outlined in the Helsinki Declaration and received approval from the Hospital Ethics Review Committee. All procedures were conducted as part of routine clinical practice. Informed consent was waived due to the retrospective nature of the study.

2.2 Data collection and 25(OH)D measurement

Demographic information, including name, gender, age, identification number, examination date, and relevant clinical data such as underlying diseases, were obtained and extracted from the hospital's electronic system. Serum 25(OH)D levels were measured using the Elecsys® Vitamin D total kit (Roche Diagnostics) on the Roche Cobas® 8,000 modular analyzer, specifically the e602 analyzer. The cutoff for defining vitamin D deficiency was based on the Clinical Application Consensus on Vitamin D and Its Analogs published by the Chinese Society of Osteoporosis and Bone Mineral Research in February 2018, which considered serum 25(OH)D levels below 20 ng/mL as indicative of deficiency.

2.3 Statistical analysis

Statistical analysis was performed using SPSS 23.0 software, and data visualization was carried out using GraphPad Prism 8 software. Non-normally distributed continuous variables were described using the median with interquartile range (IQR) and analyzed using nonparametric tests. Paired t-tests were utilized for individuals who underwent 25(OH)D testing in both years, with continuous variables presented as mean ± standard deviation. Categorical variables were analyzed using chi-square tests. Statistical significance was set at a two-tailed p-value less than 0.05.

3 Results

3.1 Distribution characteristics of the study population

A total of 1,525 healthy adult participants were enrolled from the Physical Examination Center of Henan Provincial People's Hospital for this study. The demographic characteristics and distribution of the study population are summarized in Table 1. In 2022, during the period of epidemic control, there were 479 participants (287 males, 192 females). In 2023, following the complete relaxation of epidemic measures, there were 1,046 participants (489 males, 557 females). Among the 1,525 participants, a subset of 168 individuals (101 males, 67 females) underwent continuous 25(OH)D testing for both years (2022 and 2023). Notably, there was a statistically significant difference in gender distribution between these 2 years. The collected data was stratified by age groups: 18–29 years, 30–39 years, 40–49 years, 50–59 years, and ≥ 60 years. There was no statistically significant difference in the age distribution between the populations of 2022 and 2023. However, there was a statistically significant difference in serum 25(OH)D levels and the prevalence of vitamin D deficiency between the years 2022 and 2023.

3.2 Impact of the COVID-19 pandemic on vitamin D levels in the population

Serum levels of 25(OH)D were assessed using median and quartile values in the study participants. Statistical analysis revealed a significant difference in 25(OH)D levels between the year of during COVID-19 pandemic (2022) and the year of post COVID-19 pandemic (2023), with a *p*-value of 0.004. Within the same gender group, males exhibited significantly lower serum levels of 25(OH)D in 2022 compared to 2023 ($p < 0.001$). However, no statistically significant difference was observed in 25(OH)D levels among females ($p = 0.908$). Further analysis indicated that there was no statistically significant disparity in vitamin D levels between males and females in 2022 ($p = 0.774$). Nonetheless, in 2023, the vitamin D levels among males exhibited a marked increase in comparison to females, with a statistically significant difference ($p < 0.001$) as indicated in Table 2.

Among different age groups, individuals aged 40–49 years demonstrated significantly lower serum levels of 25(OH)D in 2022 compared to 2023 ($p = 0.005$), Subsequent analysis demonstrated a statistically significant elevation in vitamin D levels among individuals aged 50–59 years and those above 60 years during both the years 2022 and 2023, in contrast to the remaining age cohorts ($p < 0.001$). The detailed results are presented in Table 3.

Furthermore, the population was stratified by month, and a comparative analysis of serum 25(OH)D levels between the 2 years was conducted. The statistical analysis revealed that the levels of 25(OH)D in February, March, and April of 2022 were significantly lower compared to the corresponding period in 2023, with *p*-values of <0.001, 0.002, and < 0.001, respectively. In contrast, the testing results in October 2022 were higher than in 2023, with a statistically significant difference ($p = 0.026$). However, there were no statistically significant differences observed in the remaining months when comparing the results between 2022 and 2023. The detailed results are presented in Table 4.

For the subset of 168 individuals who underwent continuous 25(OH)D testing over two consecutive years, the results were reported as mean ± standard deviation. The overall testing results in 2022 (20.73 ± 9.37) were significantly lower than those in 2023 (22.28 ± 8.59), with a *p*-value of 0.012. Among females, the testing results in 2022 (19.37 ± 9.60) were significantly lower than those in 2023 (21.97 ± 9.46), with a p-value of 0.015. However, among males,

TABLE 1 Characteristics of study subjects in the year 2022 (during COVID-2019 pandemic) compared to 2023 (post COVID-2019 pandemic).

Variables	2022(*n* = 479)	2023(*n* = 1,046)	*p*-value
Gender, n (%)			
Male	287(59.9%)	489(46.7%)	<0.001
Female	192(40.1%)	557(53.3%)	
Age (years)	45(36, 56)	47(35, 56)	0.429
Age (years), n (%)			
18–29	36(7.5%)	103(9.8%)	0.051
30–39	148(30.9%)	273(26.1%)	
40–49	114(23.8%)	212(20.3%)	
50–59	108(22.5%)	272(26.0%)	
≥60	73(15.2%)	186(17.8%)	
25(OH)D (ng/mL)	18.14(13.78, 23.68)	19.15(14.88, 25.01)	0.004
VD status (ng/mL), n (%)			
<20	297(62%)	574(54.9%)	0.009
≥20	182(38%)	472(45.1%)	

COVID-19, coronavirus disease 2019; 25(OH)D, 25-hydroxy vitamin D.

TABLE 2 The 25(OH)D concentration and the rate of vitamin D deficiency in different gender groups in 2022(during COVID-2019 pandemic) compared to 2023 (post COVID-2019 pandemic).

Variables	2022(*n* = 479)	2023(*n* = 1,046)	*p*-value
Total M(P25-P75)	18.14(13.78, 23.68)	19.15(14.88, 25.01)	0.004
Male M(P25-P75)	18.01(14.10, 23.53)	20.49(16.11, 26.01)	<0.001
Female M(P25-P75)	18.28(13.27, 24.39)	17.69(13.72, 23.43)	0.908
p-value	0.774	<0.001	
VD status <20 (ng/mL), n (%)			
Total	297(62%)	574(54.9%)	0.009
Male	184(64.1%)	231(47.2%)	<0.001
Female	113(58.9%)	343(61.6%)	0.505
p-value	0.245	<0.001	

The concentration unit of 25(OH)D was ng/mL. COVID-19, coronavirus disease 2019; 25(OH)D, 25-hydroxy vitamin D.

TABLE 3 The 25(OH)D concentration and the rate of vitamin D deficiency in different age groups in 2022(during COVID-2019 pandemic) compared to 2023 (post COVID-2019 pandemic).

Age (years)	2022		2023		*p*-value	2022	2023	*p*-value
	n	M (P25, P75)	n	M (P25, P75)		25(OH)D < 20 ng/mL, n (%)		
18–29	36	16.09(13.43,21.41)	103	15.08(12.81,21.26)	0.706	26(72.2%)	75(72.8%)	0.945
30–39	148	17.10(12.87,20.49)	273	17.59(13.84,22.12)	0.059	110(74.3%)	179(65.6%)	0.064
40–49	114	16.10(12.41,21.18)	212	18.28(13.91,23.86)	0.005	83(72.8%)	127(59.9%)	0.020
50–59	108	20.14(15.31,27.13)	272	21.63(16.34,27.76)	0.322	52(48.1%)	121(44.5%)	0.518
>60	73	24.20(17.16,33.70)	186	21.47(17.18,29.88)	0.200	26(35.6%)	72(38.7%)	0.644
p		<0.001		<0.001		<0.001	<0.001	

COVID-19, coronavirus disease 2019; 25(OH)D, 25-hydroxy vitamin D.

TABLE 4 The 25(OH)D serum concentration and the rate of vitamin D deficiency in each month of the year 2022 (during COVID-2019 pandemic) and 2023 (post COVID-2019 pandemic).

	2022		2023		*p*-value	2022	2023	*p*-value
	n	M (P25, P75)	n	M (P25, P75)		25(OH)D < 20 ng/mL, n (%)		
Jan	13	15.06(12.23, 18.66)	57	15.73(12.69, 21.49)	0.345	10(76.9%)	42(73.7%)	0.809
Feb	56	12.23(10.46, 14.81)	50	17.24(13.70, 23.84)	<0.001	54(96.4%)	31(62.0%)	<0.001
Mar	38	16.54(11.97, 21.90)	93	19.86(16.40, 25.28)	0.002	28(73.7%)	47(50.5%)	0.015
Apr	76	15.91(12.53, 19.49)	130	20.16(15.45, 25.46)	<0.001	61(80.3%)	70(53.8%)	<0.001
May	63	18.40(13.96, 21.93)	123	19.02(15.02, 23.85)	0.297	41(65.1%)	68(55.3%)	0.199
June	50	23.58(17.29, 29.91)	98	20.16(16.64, 27.92)	0.090	18(36.0%)	48(49.0%)	0.133
July	46	22.42(16.72, 26.60)	81	22.55(18.14, 29.36)	0.237	18(39.1%)	26(32.1%)	0.423
Aug	42	22.09(17.56, 31.75)	52	23.89(17.62, 30.26)	0.957	16(38.1%)	22(42.3%)	0.679
Sep	55	19.45(14.47, 23.70)	65	22.27(16.33, 26.86)	0.196	30(54.5%)	25(38.5%)	0.078
Oct	27	19.89(15.35, 30.50)	100	17.01(13.88, 21.64)	0.026	14(51.9%)	66(66.0%)	0.177
Nov	8	17.58(13.38, 23.30)	97	16.40(13.21, 21.77)	0.704	5(62.5%)	68(70.1%)	0.653
Dec	5	35.07(13.87, 41.58)	100	16.33(12.42, 21.16)	0.078	2(40.0%)	67(67.0%)	0.215
p		<0.001		<0.001		<0.001	<0.001	

The concentration unit of 25(OH)D was ng/mL. COVID-19, coronavirus disease 2019; 25(OH)D, 25-hydroxy vitamin D.

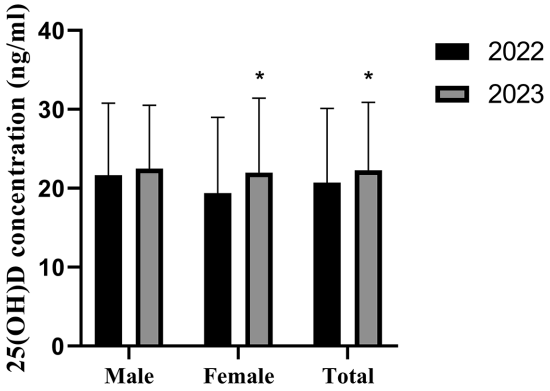

FIGURE 1
The 25(OH)D was tested in 168 participants (male, 101 and female, 67) both of the year 2022 and 2023. Error bars indicated mean ± SD, *$p < 0.05$.

there was no statistically significant difference in the testing results between 2022 (21.64 ± 9.15) and 2023 (22.49 ± 8.01), with a p-value of 0.253, as presented in Figure 1.

3.3 Impact of the COVID-19 pandemic on the prevalence of vitamin D deficiency in the population

In the year during COVID-19 pandemic in 2022, a total of 297 individuals (62% of the total population) had serum 25(OH)D levels below 20 ng/mL. In 2023, the number of individuals with vitamin D deficiency increased to 574, representing 54.9% of the total population. Statistical analysis revealed a significant difference in the prevalence of vitamin D deficiency between 2022 and 2023 ($p = 0.009$). When stratifying by gender, the prevalence of vitamin D deficiency among males was 64.1% in 2022, which was higher than the 47.2% in 2023, with a statistically significant difference ($p < 0.001$). However, no statistically significant difference was observed in the prevalence of vitamin D deficiency among females ($p = 0.505$). Further analysis demonstrated that there was no statistically significant difference in the prevalence of vitamin D deficiency between males and females in 2022 ($p = 0.245$). However, in 2023, the prevalence of vitamin D deficiency among males was significantly lower in comparison to females, with a statistically significant difference ($p < 0.001$). The results are presented in Table 2.

When comparing by age group, the prevalence of vitamin D deficiency in the 40–49 age group in 2022 was 72.8%, higher than the

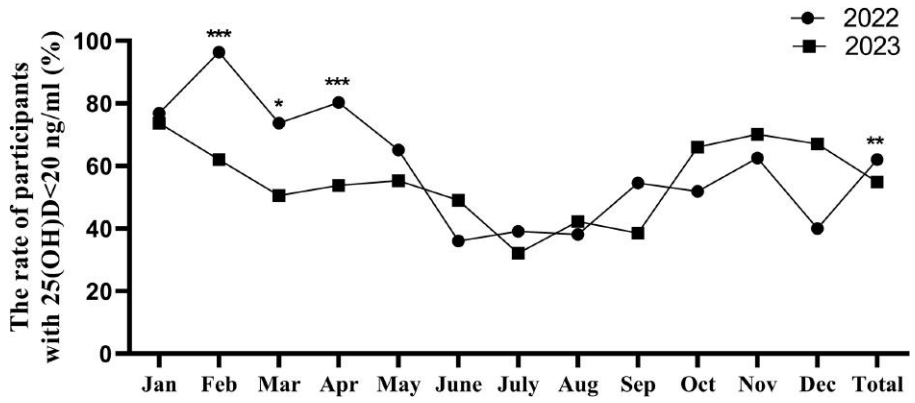

FIGURE 2
The monthly vitamin D deficiency rates for 2022 and 2023, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

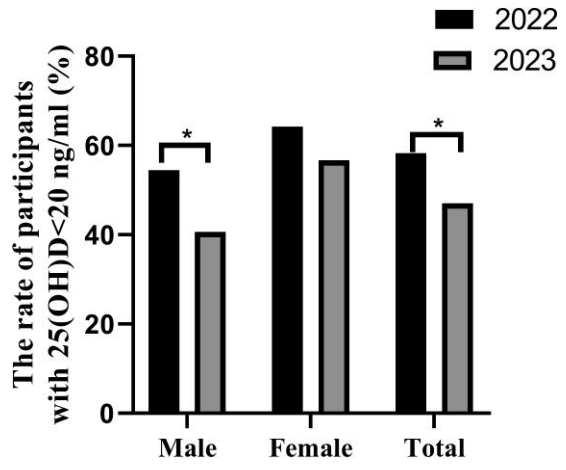

FIGURE 3
The rate of vitamin D deficiency in different gender groups in 168 participants (male, 101 and female, 67) both of the year 2022 and 2023, *$p<0.05$.

59.9% in 2023, with statistically significant differences ($p = 0.020$). No statistically significant difference was observed in the prevalence of vitamin D deficiency in other age groups. Further analysis revealed a significantly lower prevalence of vitamin D deficiency among individuals aged 50–59 years and those above 60 years in both 2022 and 2023, as compared to other age groups. This difference was statistically significant ($p < 0.001$). The results are presented in Table 3.

When comparing by month, the prevalence of vitamin D deficiency in February, March, and April of 2022 was 96.4, 73.7, and 80.3%, respectively, higher than the corresponding periods in 2023, which were 62.0, 50.5, and 53.8%, respectively, with statistically significant differences ($p < 0.001$, 0.015, and < 0.001, respectively). No statistically significant difference was observed in the prevalence of vitamin D deficiency in other months. The results are presented in Table 4 and Figure 2.

Among the 168 individuals who underwent 25(OH)D tests in both years, the prevalence of vitamin D deficiency in 2022 was 58.3%, higher than the 47.0% in 2023, with a statistically significant difference ($p = 0.038$). Among males, the prevalence of deficiency in 2022 was 54.5%, higher than the 40.6% in 2023, with a statistically significant difference ($p = 0.049$). No statistically significant difference was observed in the prevalence of vitamin D deficiency among females, as presented in Figure 3.

4 Discussion

The main findings of this study demonstrate a significant increase in overall 25-hydroxyvitamin D (25(OH)D) levels from 2022 to 2023, particularly notable in males. The prevalence of vitamin D deficiency also showed a significant decrease during this period. These findings can be attributed to the significant reduction in outdoor activities and sunlight exposure during the COVID-19 lockdown periods in 2022. Henan Province experiences substantial seasonal variations in sunlight, crucial for vitamin D synthesis. The lockdown measures enforced during the pandemic, particularly from January to February, May, and October to December 2022, coincided with periods of lower sunlight exposure, exacerbating vitamin D deficiency. Additionally, the heightened awareness of health and possible increased use of vitamin D supplements post-pandemic may have contributed to the improved vitamin D status observed in 2023.

Following the onset of the COVID-19 pandemic, China implemented stringent control strategies aimed at effectively mitigating disease transmission. These measures, however, imposed significant restrictions on individuals' mobility, with some even being confined to their residences during the peak of the outbreak. Throughout 2022, the city of Zhengzhou experienced a total of 107 days characterized by rigorous control measures, resulting in a marked reduction in outdoor activities among the populace. It was not until December 14, 2022, that these stringent control policies were abruptly lifted, leading to a rapid propagation of the virus. By January 6, 2023, the Health Commission of Henan Province reported an infection rate of 89% for COVID-19 across the province, with urban areas exhibiting a rate of 89.1% and rural areas at 88.9%. Subsequently, the daily lives of individuals returned to a state of normalcy prior to the pandemic. The principal source of vitamin D synthesis in the human body stems from direct cutaneous exposure to sunlight, particularly ultraviolet B radiation (2). Consequently, the protracted duration of containment measures in Zhengzhou, Henan during 2022 is anticipated to exert an influence on the population's vitamin D levels and the prevalence of vitamin D insufficiency.

Vitamin D plays a crucial role in the absorption of calcium which exerts pivotal roles in diverse physiological processes encompassing cellular signaling, hemostasis, myocyte contractility, and neural modulation (21). Moreover, investigations have elucidated the ability of vitamin D to facilitate phosphorus absorption, thereby directly

influencing the calcium-phosphorus balance and skeletal metabolism in the human system (22). Epidemiological evidence has firmly established associations linking vitamin D deficiency with an array of chronic ailments, including autoimmune disorders such as multiple sclerosis, type 1 diabetes, and rheumatoid arthritis, as well as cardiovascular disease, type 2 diabetes, neurocognitive impairments, and infectious diseases, notably including COVID-19 (23–26).

Henan Province is situated in the central-eastern region of China, primarily characterized by expansive plains and encompassing an area of 16.7 square kilometers. Its geographic center is located at coordinates 33.5°N and 113.3°E. The province primarily falls within the warm temperate zone, with the southern part transitioning from subtropical to warm temperate, featuring a continental monsoon climate with well-defined seasonal variations. The four seasons are delineated as follows: Winter (December to February), Spring (March to May), Summer (June to August), and Autumn (September to November). The annual average temperature in Zhengzhou, the provincial capital, is approximately 15.6°C. August is the hottest month, with a mean temperature of 25.9°C, while January is the coldest, averaging 2.15°C. The region experiences a frost-free period of roughly 209 days and an annual sunshine duration of approximately 1869.7 h.

In this study, we observed a substantial decline in the number of individuals seeking medical examinations at our institution due to the COVID-19 pandemic, particularly during January and October to December of 2022, which exhibited a significant decrease compared to the corresponding periods in 2023. Concurrently, the pandemic of COVID-19 resulted in a decline in the overall serum 25(OH)D levels and an increase in the overall prevalence of vitamin D deficiency within the adult population. When stratifying by gender, we found that the impact of the COVID-19 pandemic was more pronounced among males than females. Specifically, in males, the serum 25(OH)D levels in 2023 were significantly higher than those in 2022, and the prevalence of vitamin D deficiency in 2023 was significantly lower than that in 2022. However, no significant differences in serum 25(OH)D levels and deficiency rates were observed between the 2 years among females. When comparing genders within the same year, the serum vitamin D levels in females were slightly higher than those in males in 2022, and the prevalence of vitamin D deficiency was lower than that in males, albeit these differences did not reach statistical significance. This phenomenon may be attributable to the fact that vitamin D is a fat-soluble vitamin stored in adipose tissue, and the disparity in body fat composition between genders may play a contributory role (27). In 2023, this pattern was reversed, with significantly higher serum vitamin D levels and significantly lower deficiency rates observed among males compared to females. This intriguing finding may be elucidated by the impact of the COVID-19 pandemic, as even though both males and females experienced reduced outdoor activity, females tend to adhere more diligently to sunscreen usage. Research has demonstrated that sunscreen with SPF30 can diminish vitamin D synthesis by 95% (28). Consequently, the impact of the COVID-19 pandemic appears to have a greater effect on males than females. This phenomenon was further corroborated by the analysis of vitamin D levels in 168 individuals examined in both 2022 and 2023. The mean serum 25(OH)D levels in 2023 were higher than those in 2022, both in the overall population and when comparing the same gender. Furthermore, the prevalence of vitamin D deficiency in 2023 was lower than that in 2022. Although disparities in 25(OH)D levels were noted among females between the 2 years, no significant differences in vitamin D deficiency rates were observed. In contrast, no disparities in 25(OH)D levels were observed among males between the 2 years, but a significant difference in vitamin D deficiency rates was evident. In the comparison of different age groups, it was observed that the vitamin D levels in the 40–49 age group were significantly lower in 2022 compared to 2023. Additionally, the prevalence of vitamin D deficiency in this age group was significantly higher in 2022 than in 2023. Moreover, irrespective of the year (2022 or 2023), there was a gradual increase in vitamin D levels and a decrease in deficiency rates with advancing age. These findings are consistent with previous research conducted in various regions of China and other countries globally (29, 30). The observed pattern can potentially be attributed to increased outdoor activity among older individuals after retirement, considering that the retirement age in China is 60 for males and 50 or 55 for females. Regarding the monthly comparison, it was noted that vitamin D levels in February, March, and April of 2022 were significantly lower than the corresponding months in 2023. Similarly, the deficiency rates of vitamin D in 2022 were higher compared to 2023 during the same months. These outcomes align with previous investigations in children conducted at Henan Children's Hospital (20). Furthermore, this study revealed that vitamin D levels were highest during the summer months (June, July, August, and September) in both 2022 and 2023, followed by a gradual decline, reaching the lowest levels during the winter season. Overall, vitamin D levels during the summer and autumn seasons were significantly higher compared to the winter and spring seasons. These findings are consistent with prior studies conducted in other regions of China and other countries (30–32). The observed trends can be attributed to longer daylight hours, elevated ultraviolet radiation levels during the summer, as well as favorable weather conditions and increased outdoor activity time during the autumn season, all of which contribute to higher vitamin D levels.

This study represents the inaugural comparison of adult vitamin D levels and deficiency rates during and post the COVID-19 pandemic in Henan Province of China. Nonetheless, several limitations warrant consideration. Firstly, the study cohort primarily emanated from Henan Province, a plain region in China, thereby potentially engendering variances when juxtaposed with diverse locales. Secondly, the implementation of stringent containment measures compelled individuals to adhere to home confinement, consequently yielding a constrained sample size during this period. Notably, the study lacked comprehensive documentation pertaining to individual-specific outdoor activity duration, dietary habits, and vitamin D supplementation regimens, thereby potentially introducing inherent biases in the derived findings.

In conclusion, this study demonstrates that the COVID-19 pandemic significantly increased the proportion of vitamin D deficiency in the adult population, particularly in males. These findings underscore the importance of sufficient outdoor activities for maintaining adequate vitamin D levels.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Henan Provincial People's Hospital Ethics Review Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because informed consent was waived due to the retrospective nature of the study.

Author contributions

YC: Writing – original draft. GK: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Serum 25-hydroxyvitamin D concentrations and their impact on all-cause mortality in Parkinson's disease: insights from National Health and Nutrition Examination Survey 1999–2020 data

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Background and purpose: This study explores the relationship between serum 25-hydroxyvitamin D [25(OH)D] levels and mortality among Parkinson's disease (PD) patients, providing evidence for the potential benefits of vitamin D (VD) supplementation.

Methods: PD patients were collected from the National Health and Nutrition Examination Survey (NHANES) database from 1999 to 2020. These patients were categorized based on their serum 25(OH)D levels: deficiency, insufficiency, and sufficiency. We compared demographic information and analyzed mortality data from the National Death Index. A restricted cubic spline model assessed the nonlinear association between 25(OH)D levels and mortality, complemented by multivariable Cox regression analysis. Consistency of results was checked through subgroup analysis.

Results: The study included 364 PD patients: 87 (23.9%) with VD deficiency, 121 (33.2%) with insufficiency, and 156 (42.9%) with sufficiency. Demographically, 46.4% were male, and 56% were over 65years. The deficiency group predominantly consisted of Mexican Americans (53.1%), had lower income levels, a higher unmarried rate, and increased liver disease incidence. The analysis showed a U-shaped curve between 25(OH)D levels and mortality risk, with the lowest risk at 78.68nmol/L (p-non-linear=0.007, p-overall=0.008). Kaplan–Meier analysis found the highest survival rates in patients with 25(OH)D levels between 75–100nmol/L (p=0.039). Compared to this group, patients with levels below 50nmol/L had a 3.52-fold increased mortality risk (95% CI=1.58–7.86, p=0.002), and those above 100nmol/L had a 2.92-fold increase (95% CI=1.06–8.05, p=0.038). Age-specific subgroup analysis (p=0.009) revealed that both very low (<50nmol/L) and high (>100nmol/L) levels increased mortality risk in patients under 65, while levels below 75nmol/L raised mortality risk in older patients.

Conclusion: Serum 25(OH)D levels are nonlinearly linked to mortality in PD patients, with optimal survival rates occurring at 75–100 nmol/L. Deviations from this range increase the risk of death.

KEYWORDS
Parkinson's disease, vitamin D, all-cause mortality, NHANES, nutrition

1 Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, marked by symptoms such as resting tremor, rigidity, bradykinesia, and postural instability. The prevalence of PD has increased significantly, from 0.9 per 1,000 individuals in the 1980s to 3.81 per 1,000 in the period from 2010 to 2023. Among those over 60 years old, the incidence is 9.34 per 1,000. Estimates suggest that by 2030, the global PD population will range between 8.7 and 9.3 million (1, 2). The pathological hallmarks of PD include the accumulation of Lewy bodies and the loss of dopaminergic neurons in the substantia nigra (3). Risk factors for PD encompass older age, male sex, genetic predispositions, exposure to pesticides, concurrent diabetes, and dairy consumption (4, 5), whereas smoking, regular physical activity, and caffeine intake may offer protective effects (6). Research indicates that PD patients experience a 2.22-fold increase in all-cause mortality compared to the general population, with mortality risk increasing by 1.05 times with each additional year of age (7, 8). A report by Public Health England noted a 45% rise in PD-related mortality from 2001 to 2014 (9), highlighting the critical need to address mortality to improve outcomes for PD patients.

Vitamin D (VD), a fat-soluble vitamin, is synthesized in the skin or obtained through dietary supplements. It is converted to 25-hydroxyvitamin D [25(OH)D] in the liver and further hydroxylated in the kidneys to its active form, 1,25-dihydroxyvitamin D (10). Serum levels of 25(OH)D below 50 nmol/L typically indicate VD deficiency (11). A survey utilizing the National Health and Nutrition Examination Survey (NHANES) data in the United States found that 41.6% of adult participants had 25(OH)D levels below this threshold (12). VD deficiency impacts calcium absorption, potentially leading to osteoporosis, and is associated with higher risks of several conditions including cancer, infections, autoimmune and cardiovascular diseases, and various psychological and neurological disorders such as depression, bipolar disorder, schizophrenia, Alzheimer's disease, and PD. (13–15) The National Academy of Sciences advises a daily intake of 15 micrograms (600 IU) of VD for individuals under 70 years old and 20 micrograms (800 IU) for those aged 70 and above to mitigate these risks (16).

Previous research has shown a significant inverse relationship between serum 25(OH)D levels and PD incidence rates (17). Both VD deficiency [total 25(OH)D <50 nmol/L] and insufficiency [total 25(OH)D <75 nmol/L] increase the risk of PD development (18). However, the association between 25(OH)D levels and mortality rates in PD patients has been less studied. This research utilizes NHANES data to explore the impact of serum 25(OH)D levels on all-cause mortality in PD patients, aiming to provide insights that could improve prognosis for these patients.

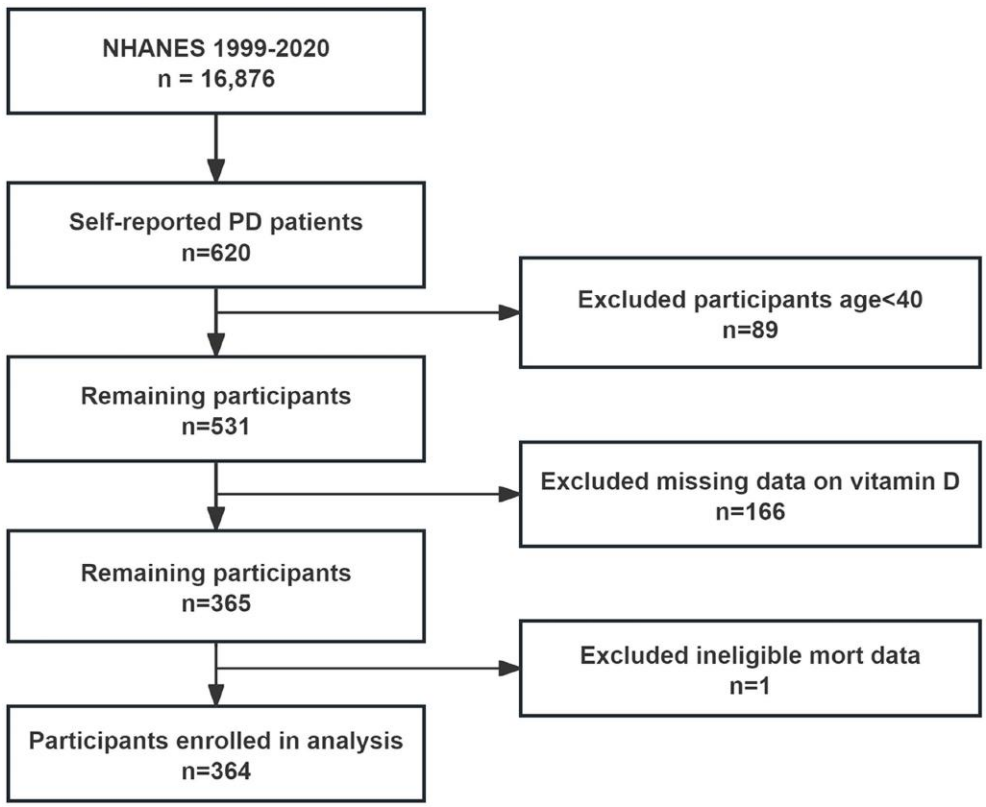

FIGURE 1
Flow chart of study inclusion.

TABLE 1 Basic characteristics of participants.

Characteristics	Deficiency (<50 nmol/L) (*n* = 87)	Insufficiency (50–75 nmol/L) (*n* = 121)	Sufficiency (≥75 noml/L) (*n* = 156)	*p*-value
Age, *n* (%)				0.4
≤65	42 (63%)	54 (59%)	64 (53%)	
>65	45 (37%)	67 (41%)	92 (47%)	
Sex, *n* (%)				0.6
Female	46 (67%)	59 (59%)	90 (64%)	
Male	41 (33%)	62 (41%)	66 (36%)	
Smoking, *n* (%)				0.065
Never	33 (43%)	62 (54%)	82 (55%)	
Former	27 (20%)	39 (27%)	49 (28%)	
Now	27 (37%)	20 (19%)	25 (16%)	
Drinking, *n* (%)				0.2
Never	11 (8.2%)	15 (10%)	20 (9.9%)	
Former	34 (38%)	39 (27%)	45 (24%)	
Now	42 (54%)	67 (63%)	91 (66%)	
Ethnicity, *n* (%)				0.002
Mexican American	17 (8.9%)	7 (2.7%)	8 (1.6%)	
Non-Hispanic White	48 (73%)	81 (81%)	119 (91%)	
Non-Hispanic Black	15 (11%)	15 (7.0%)	17 (4.0%)	
Other race	7 (7.3%)	18 (8.9%)	12 (3.5%)	
Marital status, *n* (%)				0.039
Never married	16 (16%)	15 (12%)	6 (3.0%)	
Widowed/divorced/separated	30 (33%)	48 (41%)	31 (25%)	
Married/living with partner	41 (52%)	58 (48%)	62 (72%)	
PIR, *n* (%)				0.031
<1.3	44 (38%)	43 (27%)	37 (14%)	
1.3–3.5	25 (28%)	48 (43%)	73 (46%)	
≥3.5	18 (34%)	30 (30%)	46 (40%)	
Education level, *n* (%)				0.5
Less than high school	35 (26%)	36 (21%)	39 (16%)	
High school/equivalent	16 (23%)	29 (26%)	30 (22%)	

(Continued)

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TABLE 1 (Continued)

Characteristics	Deficiency (<50 nmol/L) (*n* = 87)	Insufficiency (50–75 nmol/L) (*n* = 121)	Sufficiency (≥75 noml/L) (*n* = 156)	*p*-value
College/more than high school	36 (51%)	56 (53%)	87 (63%)	
BMI, kg/m^2, *n* (%)				0.4
<25	23 (20%)	33 (26%)	44 (31%)	
25–28	22 (32%)	35 (26%)	50 (32%)	
≥28	42 (48%)	53 (47%)	62 (37%)	
Caffeine consumption	70 (11, 140)	126 (28, 243)	133 (50, 245)	0.11
Physical activity				0.12
Active	15 (28%)	35 (47%)	46 (34%)	
Inactive	57 (72%)	65 (53%)	93 (66%)	
Diabetes mellitus, *n* (%)				0.5
No	54 (69%)	88 (77%)	108 (76%)	
Yes	33 (31%)	33 (23%)	48 (24%)	
Hyperlipidemia, *n* (%)				0.7
No	21 (24%)	21 (18%)	26 (17%)	
Yes	66 (76%)	100 (82%)	130 (83%)	
Hypertension, *n* (%)				0.3
No	30 (34%)	38 (32%)	51 (42%)	
Yes	57 (66%)	83 (68%)	105 (58%)	
Stroke, *n* (%)				0.4
No	71 (81%)	103 (88%)	139 (90%)	
Yes	16 (19%)	18 (12%)	17 (9.8%)	
ASCVD, *n* (%)				0.06
No	56 (66%)	80 (75%)	120 (84%)	
Yes	31 (34%)	41 (25%)	36 (16%)	
Cancer, *n* (%)				0.6
No	69 (70%)	94 (75%)	124 (78%)	
Yes	18 (30%)	27 (25%)	32 (22%)	
Chronic heart failure, *n* (%)				0.7
No	76 (86%)	107 (91%)	140 (92%)	
Yes	11 (14%)	14 (9.2%)	16 (8.2%)	

(Continued)

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TABLE 1 (Continued)

Characteristics	Deficiency (<50 nmol/L) (*n* = 87)	Insufficiency (50–75 nmol/L) (*n* = 121)	Sufficiency (≥75 noml/L) (*n* = 156)	*p*-value
COPD, *n* (%)				0.5
No	80 (91%)	104 (85%)	138 (90%)	
Yes	7 (9.2%)	17 (15%)	18 (10%)	
Liver disease, *n* (%)				0.019
No	78 (86%)	117 (98%)	143 (90%)	
Yes	9 (14%)	4 (2.2%)	13 (9.5%)	

PIR, ratio of family income to poverty; BMI, body mass index; ASCVD, arteriosclerotic cardiovascular disease; COPD, chronic obstructive pulmonary disease.

TABLE 2 Cause of death in participants.

Characteristic	Overall (*n* = 132)	Deficiency (<50 nmol/L) (*n* = 39)	Insufficiency (50–75 nmol/L) (*n* = 50)	Sufficiency (≥75noml/L) (*n* = 43)	*p*-value
Cause of death					0.5
Diseases of heart	34 (24%)	8 (22%)	13 (25%)	13 (25%)	
Malignant neoplasms	19 (14%)	8 (24%)	6 (14%)	5 (7.3%)	
Chronic lower respiratory diseases	4 (2.2%)	1 (4.4%)	3 (3.0%)	0 (0%)	
Accidents	4 (3.0%)	0 (0%)	1 (1.7%)	3 (6.3%)	
Cerebrovascular diseases	4 (2.7%)	2 (2.5%)	1 (1.0%)	1 (4.5%)	
Alzheimer's disease	6 (4.0%)	2 (6.9%)	3 (4.9%)	1 (1.4%)	
Diabetes mellitus	3 (3.5%)	1 (3.5%)	1 (3.8%)	1 (3.2%)	
Influenza and pneumonia	3 (2.9%)	1 (4.1%)	2 (5.1%)	0 (0%)	
Nephritis, nephrotic syndrome and nephrosis	1 (0.3%)	0 (0%)	0 (0%)	1 (0.7%)	
All other causes	54 (43%)	16 (33%)	20 (41%)	18 (51%)	

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2 Materials and methods

2.1 Study design and participants

The NHANES database, managed by the National Center for Health Statistics (NCHS), assesses the health and nutritional status of the United States population. This study obtained Institutional Review Board approval from the NCHS, with all participants providing written informed consent.

2.2 Diagnosis of PD

Participants from the NHANES questionnaire survey who reported using medications such as methyldopa, levodopa, carbidopa, pramipexole, ropinirole, amantadine, selegiline, entacapone, benztropine, trihexyphenidyl, zonisamide, apomorphine, tolcapone, orphenadrine, rasagiline, rotigotine, and safinamide, based on criteria established in previous studies (19–21).

2.3 Measurement of serum 25(OH)D

Serum levels of 25(OH)D, including both D2 and D3 variants, were initially measured using the RIA method (NHANES 2001–2006) and subsequently by standardized LC-MS/MS (2007–2018). Data from 2001–2006 were adjusted to align with LC-MS/MS standards for consistency. VD status was classified into three categories: deficiency (<50 nmol/L), insufficiency (50–75 nmol/L), and sufficiency (≥75 nmol/L) (10).

2.4 Ascertainment of mortality

Mortality data were sourced from the National Death Index (NDI) and updated through December 31, 2019. The data were linked via the NCHS, with mortality eligibility indicated by the variable ELIGSTAT and vital status by MORTSTAT. Follow-up duration was calculated in person-months from the interview date to either the date of death or the end of the study, tracked by the variable PERMTH_INT. Causes of death were classified according to the 10th revision of the International Statistical Classification of Diseases, Injuries, and Causes of Death (ICD-10).

TABLE 3 Relationship between serum 25(OH)D and the all-cause mortality.

Vitamin D	HR	95% CI	*p*-value
Unadjusted	0.99	0.98–1.00	0.200
Model1	0.99	0.98–1.00	0.243
Model2	0.99	0.98–1.01	0.271

Model 1 was adjusted for age, sex, marital status, smoking, drinking, BMI. Model 2 was adjusted for Model 1 + diabetes mellitus, hypertension, COPD, chronic heart failure, stroke, ASCVD, cancer. HR, hazard ratio; CI, confidence intervals; BMI, body mass index; ASCVD, arteriosclerotic cardiovascular disease.

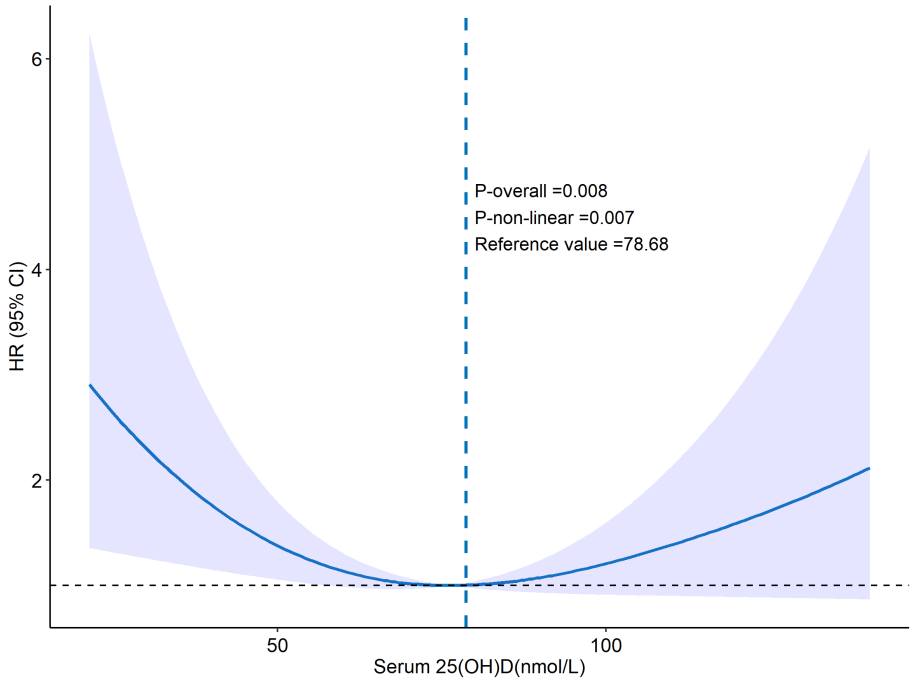

FIGURE 2
The RCS curve of 25(OH)D and all-cause mortality. Adjusted by age, sex, marital status, ethnicity, smoking, PIR, education level, drinking, BMI, diabetes mellitus, hypertension, hyperlipidemia, COPD, chronic heart failure, stroke, ASCVD, cancer, liver disease.

2.5 Assessment of covariates

Demographic information and medical history were collected to serve as covariates. These included age (grouped as ≤65 years and >65 years), sex, body mass index (BMI categorized as <25, 25–30, and ≥30 kg/m^2), race, education level, marital status, and poverty income ratio (PIR categorized as <1.3, 1.3–3.5, and ≥3.5). Lifestyle factors assessed included caffeine consumption, smoking history (classified into non-smokers (less than 100 cigarettes), former smokers (more than 100 cigarettes but quit), and current smokers (more than 100 cigarettes and currently smoking) based on lifetime cigarette consumption), and alcohol consumption history. Physical activity was determined through a questionnaire, defining moderate activity as at least 10 min of exercise per day over the last 30 days that induced light sweating or a slight to moderate increase in breathing or heart rate.

Health conditions were meticulously recorded. Diabetes was identified by fasting blood glucose levels ≥7 mmol/L, HbA1c ≥11.1 mmol/L, use of diabetes medication, or a previous diagnosis by a doctor. Hypertension was defined by blood pressure ≥140/90 mmHg, use of antihypertensive medication, or a prior diagnosis. The presence of stroke, atherosclerotic cardiovascular disease (ASCVD), liver disease, chronic obstructive pulmonary disease (COPD), cancer, and chronic heart failure were all established based on self-reported medical history.

2.6 Statistical analysis

Continuous variables were presented as either medians with interquartile ranges (IQR) or means with standard deviations (SD), and analyzed using the Kruskal–Wallis test. Categorical variables were reported as frequencies and percentages and evaluated using the chi-square test. To handle missing data, multiple imputation techniques were employed.

In the analysis, serum 25(OH)D levels were treated as a continuous variable in Cox regression to assess their linear relationship with mortality. Restricted cubic spline (RCS) curves depicted this relationship, with reference groups selected based on RCS outcomes. Kaplan–Meier survival curves were generated to compare survival rates across different groups using the Breslow test.

Covariates such as age, sex, marital status, race, PIR, education level, and smoking status were included in the multivariable Cox regression analysis (Model 1). Model 2 extended adjustments to include hypertension, diabetes, stroke, ASCVD, and liver disease history, exploring the independent impact of serum 25(OH)D on PD mortality rates.

Subgroup analyses based on age and sex were conducted to ensure result consistency, employing likelihood ratio tests to explore interactions between these subgroups and 25(OH)D levels. All statistical analyses were performed using R software version 4.3.1. A *p*-value of less than 0.05 was considered statistically significant.

3 Results

3.1 Participant demographics

From the NHANES interviews conducted between 1999 and 2020, 16,876 participants were initially considered, including 620 who self-reported having PD. After excluding 89 individuals under 40 years old and 167 lacking complete serum 25(OH)D and mortality data, 364 participants were included in the final analysis (Figure 1). These

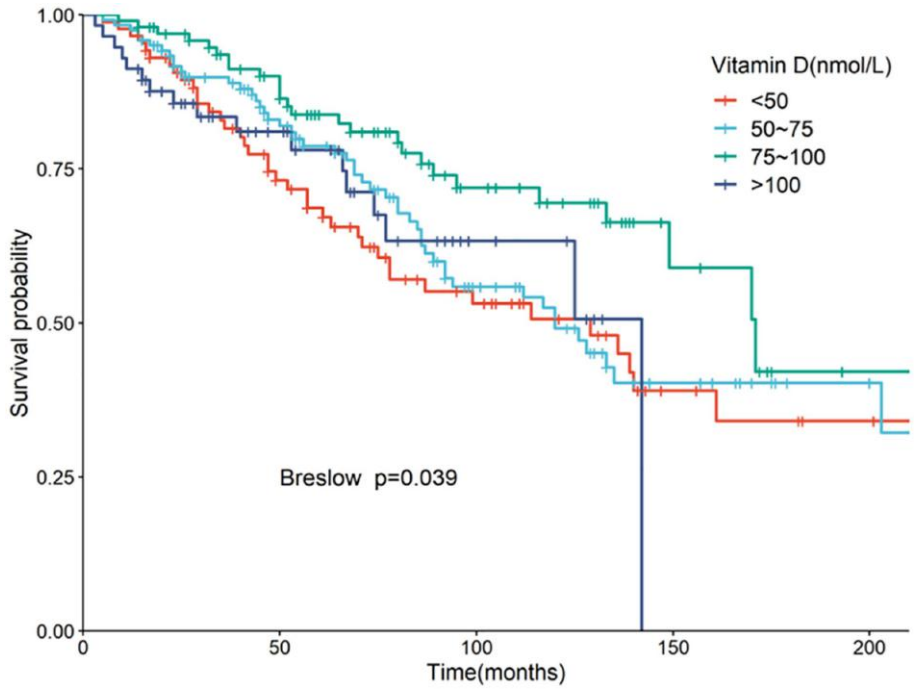

FIGURE 3
Kaplan–Meier curve of all-cause mortality by four groups of 25(OH)D.

TABLE 4 Relationship between covariant variables and the all-cause mortality.

Variables	HR (95% CI)	*p*-value
Age (>65)	3.81 (2.33–6.23)	<0.001
Male	2.12 (1.30–3.44)	0.002
Smoking		
Former	1.96 (1.11–3.45)	0.021
Now	1.54 (0.78–3.06)	0.2
Drinking		
Former	0.89 (0.42–1.90)	0.8
Now	0.6 (0.29–1.24)	0.2
Ethnicity		
Non-Hispanic White	0.99 (0.49–2.03)	>0.9
Non-Hispanic Black	0.8 (0.33–1.93)	>0.6
Other race	0.54 (0.14–2.07)	0.4
Marital status		
Widowed/divorced/separated	1.35 (0.68–2.69)	0.4
Married/living with partner	0.6 (0.30–1.20)	0.15
PIR, *n* (%)		
1.3–3.5	0.83 (0.51–1.36)	0.5
≥3.5	0.73 (0.41–1.31)	0.3
Education level		
High school or equivalent	0.41 (0.20–0.85)	0.017
College or more than high school	0.71 (0.43–1.17)	0.2
BMI		
25–28	1.61 (0.89–2.91)	0.12
≥28	0.92 (0.51–1.64)	0.8
Caffeine consumption	1.00 (1.00–1.00)	0.93
Physical activity	0.64 (0.36–1.16)	0.143
Diabetes mellitus, *n* (%)	2.18 (1.32–3.60)	0.002
Hyperlipidemia, *n* (%)	1.22 (0.71–2.07)	0.5
Hypertension, *n* (%)	2.24 (1.35–3.70)	0.002
Stroke, *n* (%)	1.85 (1.02–3.35)	0.041
ASCVD, *n* (%)	2.54 (1.55–4.17)	<0.001
Cancer, *n* (%)	1.15 (0.69–1.90)	0.6
Chronic heart failure, *n* (%)	1.87 (0.92–3.80)	0.085
COPD, *n* (%)	1.66 (0.83–3.34)	0.2
Liver disease, *n* (%)	1.73 (0.78–3.85)	0.2

PIR, ratio of family income to poverty; BMI, body mass index; ASCVD, arteriosclerotic cardiovascular disease; COPD, chronic obstructive pulmonary disease.

participants were categorized based on their serum 25(OH)D levels into deficiency (87 participants, 23.9%), insufficiency (121 participants, 33.2%), and sufficiency (156 participants, 42.9%). Analysis revealed no significant age or gender differences across these groups. Notably, the deficiency group had a higher proportion of Mexican American participants (53.1%), lower poverty income ratios, a greater number of unmarried individuals, and a higher incidence of liver disease (Table 1).

3.2 25(OH)D levels and mortality in PD

Over an average follow-up of 8.5 years, 132 PD patients died, primarily from heart disease (24%) and malignant tumors (14%). No significant mortality differences were observed among patients with varying levels of VD (Table 2). Analysis using serum 25(OH)D as a continuous variable in both univariate and multivariate Cox regression revealed no direct correlation with mortality rates among PD patients

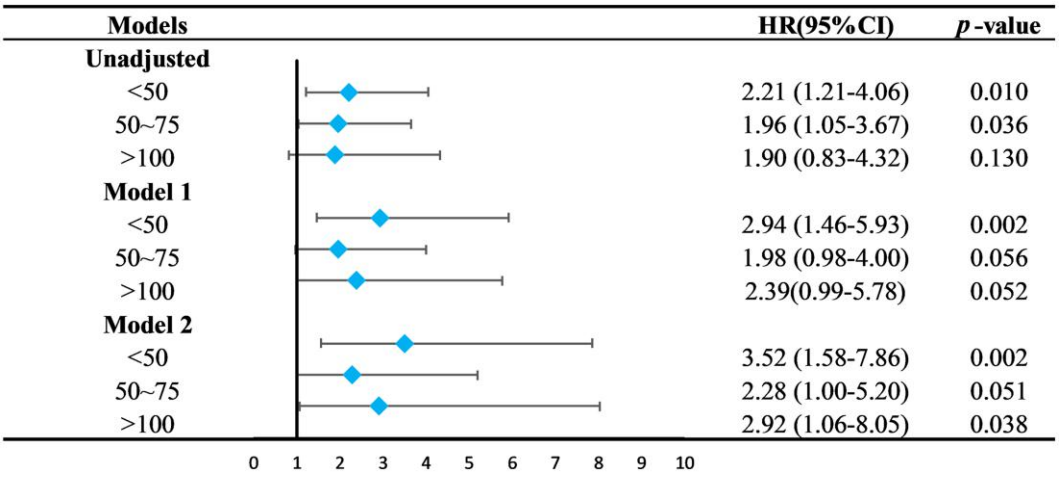

FIGURE 4
Relationship between 25(OH)D groups and the all-cause mortality. The 75–100 mmol/L group was set as the control group. Model 1 was adjusted for age, sex, marital status, ethnicity, smoking, PIR, education level. Model 2 was adjusted for Model 1 + diabetes mellitus, hypertension, stroke, ASCVD, liver disease. HR, hazard ratio; CI, confidence intervals; PIR, ratio of family income to poverty; ASCVD, arteriosclerotic cardiovascular disease.

(Table 3). However, after adjusting for variables such as age, sex, race, marital status, PIR, BMI, smoking habits, and medical conditions including hypertension, diabetes, and stroke, a U-shaped relationship emerged between serum 25(OH)D levels and mortality risk, with the lowest risk observed at 78.68 nmol/L (p-non-linear = 0.007, p-overall = 0.008) (Figure 2).

Serum 25(OH)D was grouped into four categories: Group 1 (<50 nmol/L), Group 2 (50–75 nmol/L), Group 3 (75–100 nmol/L, the reference group), and Group 4 (>100 nmol/L). Kaplan–Meier analysis showed the highest survival rate in Group 3, with significant differences noted ($p = 0.039$) (Figure 3). Univariate Cox regression analysis results, detailed in Table 4, highlighted the mortality risks associated with different serum 25(OH)D levels. Multivariate COX regression analysis results are shown in Figure 4. Model 1, adjusted for demographic factors like age and sex, indicated that the mortality risk in Group 1 was 2.94 times higher than in Group 3 (95% CI = 1.46–5.93, $p = 0.002$). Model 2 incorporated additional adjustments for covariates including hypertension, diabetes, and history of ASCVD. This model showed that the mortality risk for Group 1 was 3.52 times higher than that of Group 3 (95% CI = 1.58–7.86, $p = 0.002$), and the risk for Group 4 was 2.92 times higher (95% CI = 1.06–8.05, $p = 0.038$).

3.3 Subgroup analysis

Exploratory subgroup analyses indicated significant interactions between age and serum 25(OH)D levels ($p = 0.009$). In the subgroup aged ≤65 years, those in Groups 1 and 4 showed increased mortality risks with hazard ratios (HR) of 4.54 (95% CI = 1.29–16.0, $p = 0.018$) and 9.82 (95% CI = 2.45–39.3, $p = 0.001$), respectively. Among participants older than 65, increased risks were found in Groups 1 and 2, with HRs of 2.17 (95% CI = 1.14–4.13, $p = 0.018$) and 1.94 (95% CI = 1.11–3.40, $p = 0.02$). No significant interactions were observed for sex or comorbidities (Figure 5).

4 Discussion

This study established a U-shaped nonlinear relationship between serum 25(OH)D levels and mortality among PD patients, identifying the lowest mortality risk at approximately 78.68 nmol/L. PD patients with serum 25(OH)D concentrations within the optimal range of 75–100 nmol/L exhibited the highest survival rates. In contrast, those with levels below 50 nmol/L or above 100 nmol/L faced increases in mortality risk by factors of 3.52 and 2.92, respectively.

VD, a fat-soluble nutrient, undergoes transformation in the bloodstream to 1,25-dihydroxyvitamin D [1,25(OH)2D] through the action of 1α-hydroxylase, interacting with the vitamin D receptor (VDR) to influence gene transcription. Immunohistochemistry studies indicate a significant presence of 1α-hydroxylase and VDR in the dopaminergic neurons of the substantia nigra (22). VD plays a vital role in neuroprotection by promoting nerve growth factor production, regulating T-cell generation, reducing microglial activation, and decreasing the release of inflammatory factors, which helps in preventing the degeneration of dopaminergic neurons (23, 24).

Further supporting the importance of 25(OH)D, a Finnish cohort study reported a 65% reduced risk of developing PD in individuals with serum levels ≥50 nmol/L compared to those below 25 nmol/L. (17) Additionally, there is a correlation between 25(OH)D levels and PD motor symptoms. As PD progresses, indicated by higher Hoehn and Yahr stages, serum 25(OH)D levels tend to decrease, and there is a significant negative correlation with the Unified Parkinson's Disease Rating Scale (UPDRS) scores (22, 25). Higher levels of 25(OH)D are also linked to better cognitive and mood outcomes in PD patients (26).

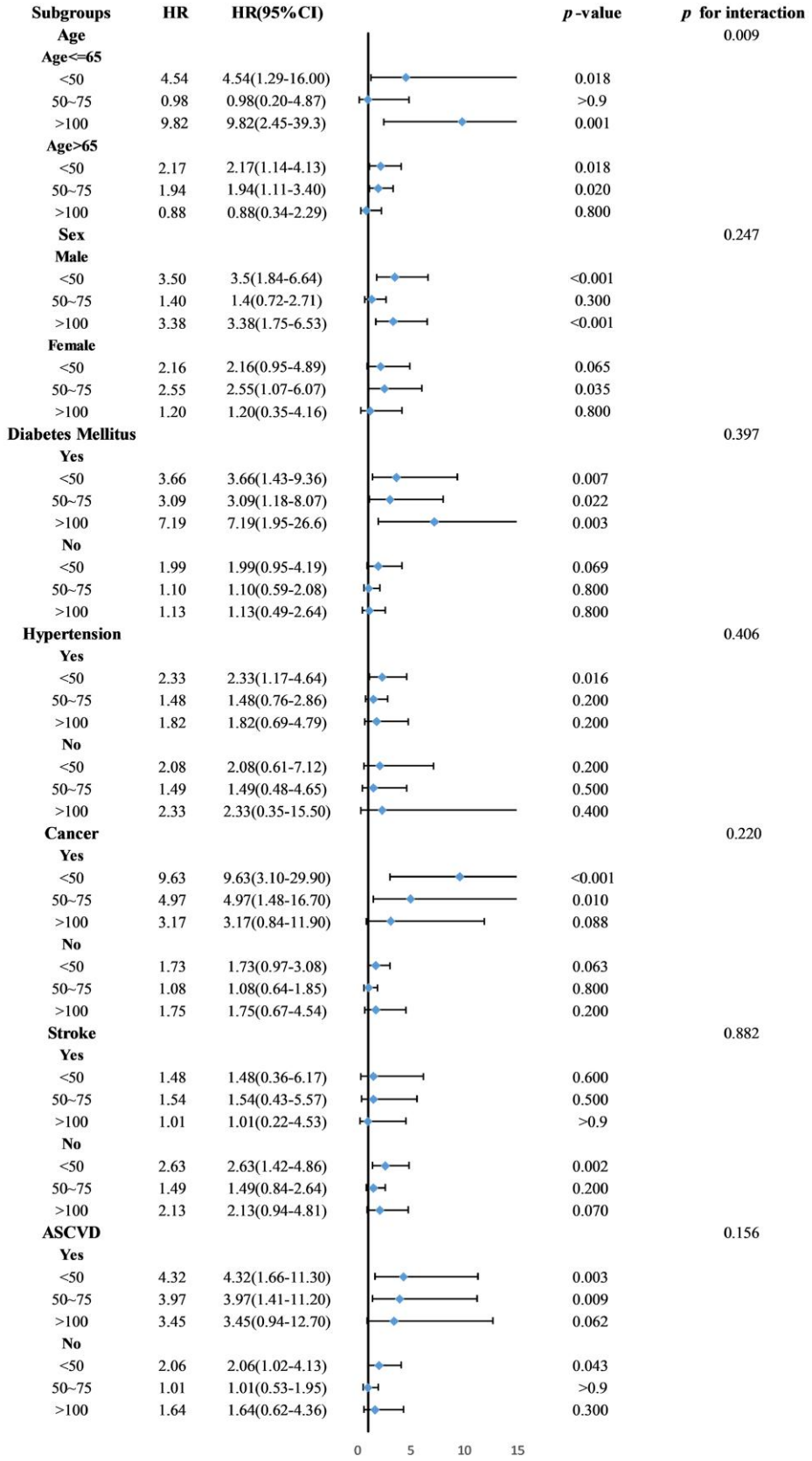

FIGURE 5
Subgroup analysis of the association between 25(OH)D and mortality.

Serum 25(OH)D is derived from synthesis in the skin following sunlight exposure and from absorption through the gastrointestinal tract. Traditionally, it was believed that reduced outdoor activity led to insufficient 25(OH)D synthesis in PD patients. However, recent findings reveal a negative correlation between 25(OH)D2, primarily absorbed via the gastrointestinal tract and independent of sunlight, and the incidence of PD. This indicates that low serum 25(OH)D levels in PD patients may also stem from gastrointestinal dysfunction, affecting nutrient absorption (18). These insights suggest potential benefits from increasing oral VD supplementation in PD patients. Indeed, a 2023 cohort study noted that oral VD comprises 71% of dietary supplements used by PD patients, yet clinical guidelines for VD supplementation in this group remain undefined (27).

Previous guidelines on optimal serum 25(OH)D levels have varied. The Institute of Medicine suggests a level of 50 nmol/L suffices for 97.5% of the population, whereas the American Geriatrics Society recommends at least 75 nmol/L for older adults (16, 28).

Numerous studies have discussed appropriate serum 25(OH)D concentrations for different populations. A meta-analysis of 14 cohort studies has identified a U-shaped relationship between serum 25(OH)D concentrations and total mortality in the general population, pinpointing 75–87.5 nmol/L as the optimal range. Beyond this, the mortality reduction becomes insignificant (29). Studies in specific demographics, such as elderly men and postmenopausal women, have shown that mortality rates escalate with levels below 46 nmol/L or above 98 nmol/L, and with levels below a cutoff of 73.89 nmol/L, respectively (30, 31). Our research uniquely demonstrates that PD patients achieve the highest survival rates with serum 25(OH)D concentrations between 75–100 nmol/L, suggesting that levels outside this range could elevate the risk of mortality.

VD functions to mitigate excitotoxic damage by reducing cytoplasmic Ca^{2+} levels, decreasing nitric oxide synthase production, and lowering the formation of free radicals and reactive oxygen species (ROS). It also modulates immune responses by down-regulating cytokines, including interleukin-2 and tumor necrosis factor-α (32). Furthermore, increased serum 25(OH)D levels are linked to longer leukocyte telomere length (LTL), which is associated with longevity (33). VD enhances the apoptosis inhibitor Bcl-2, exerting anti-apoptotic effects by inhibiting death receptor-mediated apoptosis (34). Thus, it may decrease mortality through anti-inflammatory actions, modulation of LTL, and apoptotic signaling pathways. In subgroup analyses, age-related differences were observed concerning serum 25(OH)D levels and mortality risks in PD patients. Individuals aged ≤65 years with serum 25(OH)D concentrations below 50 nmol/L or above 100 nmol/L exhibited increased mortality risks. Conversely, in those over 65 years, levels below 75 nmol/L were associated with higher mortality, whereas levels above 100 nmol/L did not significantly impact mortality. These findings could guide VD supplementation strategies across various age groups.

This study has several limitations. This study, utilizing NHANES data, primarily reflects the health status of the American population, and results may not directly apply to other demographics. The public nature of the database limits access to detailed clinical assessments like UPDRS scores or Hoehn Yahr grading of PD patients, precluding more detailed analysis across different PD stages. Moreover, while the study controlled for known confounders, unidentified interfering factors cannot be completely ruled out. As an observational study, it does not establish causality between serum 25(OH)D levels and mortality in PD patients; thus, further higher-level research is needed to confirm these observations.

5 Conclusion

Findings from this study suggest that maintaining serum 25(OH)D levels within 75–100 nmol/L optimizes outcomes for PD patients. Those with levels below this range should consider increasing their VD intake to reach the recommended levels, while those above 100 nmol/L should adjust to maintain within this optimal range.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YY: Writing – review & editing, Writing – original draft. HD: Writing – review & editing, Supervision, Conceptualization. ZZ: Writing – review & editing, Data curation, Formal analysis, Validation. YZ: Writing – original draft, Data curation, Formal analysis, Validation. MG: Writing – original draft, Investigation, Formal analysis. WL: Writing – original draft, Resources, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The relationship between physical activity levels and serum vitamin D levels varies among children and adolescents in different age groups

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Objective: The objective of the present study was to explore the relationship between physical activity (PA) levels and serum vitamin D levels in children and adolescents of different ages and sexes.

Methods: All the data in this study were collected during two cycles (2011–2014) of the National Health and Nutrition Examination Survey (NHANES). Our study participants were aged ≥3 and < 20 years and had valid data for all variables, including vitamin D intake, serum vitamin D levels, PA volume and intensity levels, amount of time spent outdoors, body mass index (BMI), sex, and race.

Results: A total of 3,312 participants were included in the study; 1,672 were boys (50.4%), and 1,640 were girls (49.6%). A total of 250 (7.5%) children were aged 3–5 years, 1,474 (44.5%) were aged 6–11 years, and 1,588 (47.9%) were aged 12–19 years. Both PA volume and intensity were positively related to serum vitamin D levels in the 6–11-year-old boys and girls ($p < 0.05$ for both) and in the 12–19-year-old boys. No significant relationship between PA volume or intensity and serum vitamin D levels was detected in the 3–5-year-old group or in the 12–19-year-old girl group. The time spent outdoors and the BMI of the participants had mediating effects on the relationships of PA volume and intensity with serum vitamin D levels in boys and girls aged 6–11 years.

Conclusion: The relationship between PA and vitamin D varies among children and adolescents of different sexes and ages, and the sun exposure level and BMI had mediating effects on the relationship between PA and the serum vitamin D level. The mechanism of the relationship between PA and increased serum vitamin D levels needs further in-depth research.

KEYWORDS

physical activity, vitamin D, children, adolescents, different ages

Introduction

Vitamin D controls plasma calcium levels after intestinal absorption, helping to regulate bone metabolism (1). In addition to calcium metabolism, vitamin D plays an important role in exoskeleton tissues such as pancreatic tissue, adipocytes, and skeletal muscle and is involved in regulating immune responses (2). Due to rapid skeletal growth and the development of

many organ systems during childhood and adolescence, vitamin D levels may be particularly important during this period.

However, vitamin D deficiency has been reported worldwide. Akkermans et al. (3) studied 325 children in Western Europe and reported that the overall prevalence of vitamin D deficiency was 22.8%. The prevalence of vitamin D insufficiency in Iranian children and adolescents was 31% (4). A multicenter, hospital-based, cross-sectional observational study from China surveyed 465,337 children from 825 hospitals in 18 provinces and reported that the prevalence rates of vitamin D deficiency (<30 nmol/L) and insufficiency (30–50 nmol/L) were 6.69 and 15.92%, respectively (5).

Vitamin D can be obtained from exposure to UV radiation from the sun, the diet, or supplements. The main source of vitamin D for humans is exposure of the skin to ultraviolet B (UVB) radiation (290–315 nm) from the sun. (6) Skin synthesis is estimated to provide 80–100% of the body's vitamin D requirements (7). However, excessive sun exposure can increase the risk of skin aging and skin cancer (8); moreover, several factors that hinder year-round synthesis, such as season, latitude, major weather conditions (9), and sunscreen use (9), have been identified.

Diets contain relatively low levels of vitamin D. Many studies have shown that vitamin D supplements and vitamin D-fortified foods significantly improve vitamin D status (10). However, only consumers obtain the benefits of supplements (11, 12). Moreover, incorrect supplementation methods, such as intermittent and high-dose treatment, may lead to unexpected adverse reactions (10).

In recent years, many studies have shown that physical activity (PA) is associated with an increase in adult vitamin D levels, excluding the impact of sunlight exposure on vitamin D levels (13, 14). Although the ability of sunlight to synthesize vitamin D decreases with age, studies targeting elderly people have shown the same results (13). Several studies have also shown a positive correlation between vitamin D levels and PA levels in children and adolescents (15, 16).

Physical activity is any movement caused by muscle contraction, which leads to an increase in energy expenditure compared to that at rest (17). PA has a positive impact on health (18). Research has confirmed that in the National Health and Nutrition Examination Survey (NHANES) population, individuals who engage in PA have a lower mortality rate (18). In the adult population, a large amount of research evidence supports the positive role of exercise interventions in improving human metabolic parameters, including lipid status, insulin resistance markers, and the levels of other related hormones (219). Research on the child population has also confirmed the role of exercise training in reducing insulin resistance (19, 20) and improving cardiac metabolic health (21, 22). Therefore, the use of PA to improve vitamin D status will provide additional and profound benefits.

However, is the relationship between PA and vitamin D affected by the physiological differences between boys and girls, the varying physical abilities at different ages, and the many physical changes that occur during adolescence? What is the relationship between PA and vitamin D levels in children of different ages and sexes? Answering these questions will provide more scientific and specific guidance for children and adolescents to adopt measures to improve vitamin D status through PA. To our knowledge, little research has been conducted on the relationship between PA and vitamin D levels in individuals of different ages and sexes. Therefore, studies exploring the relationship between PA and vitamin D levels in children and adolescents of different sexes and ages and those recommending an appropriate mode to increase vitamin D levels are necessary.

Therefore, we used data from the NHANES, which uses precise motion accelerometers to record the amount and intensity of PA, to explore the effects of PA on serum vitamin D levels in preschoolers, schoolchildren, and adolescents, which can provide a scientific basis for the study of PA in improving children's vitamin D levels according to sex and age.

Materials and methods

Study population

In this study, all data were derived from the NHANES, a nationwide assessment that evaluates the health and nutritional status of both adults and children in the United States. The NHANES uses a stratified, multistage random sampling approach to ensure representative sampling. The survey data are updated and made publicly accessible every 2 years. For this research, we analyzed data from two consecutive NHANES cycles spanning the years 2011 to −2014. Our study participants were children and adolescents aged ≥3 and < 20 years who had valid data for serum vitamin D and PA levels. The NHANES protocol was approved by the National Center for Health Statistics Research Ethics Review Board (Protocol #2011-17).

Variables

Physical activity

In 2011–2014, the NHANES utilized ActiGraph GT3X+ wrist-worn accelerometers (Pensacola, FL, United States) to assess physical activity. These devices captured triaxial acceleration data along the *x*-, *y*-, and *z*-axes. The data were translated into Monitor-Independent Movement Summary (MIMS) units on a minute-by-minute basis, employing a universal, device-agnostic algorithm. This approach facilitated consistent comparisons across diverse studies and research designs (23). The data collected on the first and last partial days were excluded from the analysis before any of the MIMS metrics were calculated (24). We excluded "nonwear" and "unknown" minutes, including only "wear" ("wake" and "sleep") minutes. Participants who wore the device for more than 10 wear hours ("wake" and "sleep") per day, who had no less than 3 "sleep" wear hours and 7 "wake" wear hours, respectively, and who wore it for at least 3 valid days were included (24).

In the present study, the MIMS metrics encompassed both PA volume, represented as the average daily MIMS units, reflecting the total number of MIMS units accumulated daily across valid assessment days, and PA intensity, which is quantified by the peak 60-min MIMS value (23). The peak 60-min MIMS value was defined as the average movement per day, encompassing the 60 highest MIMS units per minute (not necessarily consecutive) across all valid assessment days. This metric was calculated by first ranking an individual's MIMS units per minute for each valid day, determining the mean of the top 60 values within each day, and finally averaging these per-minute MIMS units across all valid wear days (24). The use of the peak 60-min MIMS value is consistent with the daily guidelines for moderate-to-vigorous

aerobic PA among children and adolescents (25) and partly mirrors the peak 60-min stepping cadence employed in previous studies (23).

Vitamin D

The serum vitamin D level (nmol/L) was determined in this study by summing the 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 levels. Ultrahigh-performance liquid chromatography–tandem mass spectrometry was utilized for the quantitative detection of vitamin D levels. The laboratory procedure manual outlines the methodologies adopted for collecting, transporting, storing, and analyzing vitamin D samples (26, 27).

Covariates

The demographic variables included age, sex, race (categorized as Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other), and the family poverty–income ratio (PIR). Additionally, body mass index (BMI) was computed by dividing an individual's weight in kilograms by the square of their height in meters. The weight status of children under 20 years of age was evaluated using the 2000 Centers for Disease Control and Prevention (CDC) growth charts, where obesity was defined as a BMI equal to or surpassing the 95th percentile of the sex-specific BMI-for-age percentiles, overweight encompassed a BMI ranging from the 85–95th percentiles, and normal weight corresponded to a BMI falling between the 5th and 85th percentiles (28)Furthermore, the total intake of vitamin D and the amount of time spent outdoors are considered two potential factors influencing serum vitamin D levels. Total vitamin D intake is derived from daily food and vitamin supplements. Individuals who consumed more than 100 μg of vitamin D per day were excluded. The amount of time spent outdoors (outdoor time) was measured through ambient light levels recorded by ActiGraph model GT3X+ accelerometers. The time spent in outdoor and indoor locations was determined by considering lux values, where ≥240 lux indicates outdoor locations and < 240 lux indicates indoor locations (27). Notably, a previous study demonstrated that a threshold of 240 lux achieved a remarkable 97% accuracy in distinguishing between indoor and outdoor conditions in a naturalistic setting (27). The daily outdoor time for each individual, reported in minutes per day, was calculated by averaging the outdoor minutes across valid wear days. Three participants were excluded because they spent more than 7 h outdoors per day, which was more than 4 times the mean daily amount of time spent outdoors.

Statistical analysis

Aggregate statistics were generated for the outcomes, exposures, and relevant covariates. Normally distributed continuous variables are presented as the means ± SDs, whereas nonnormally distributed continuous variables are presented by as medians (IQRs). First, we employed the Kruskal–Wallis rank sum test and the chi-square test to assess the subgroup differences according to sex (male and female) and age group (3–5, 6–11, and 12–19 years) in the descriptive analysis of the baseline characteristics of the participants. Stratified multivariate linear regressions were performed to analyze the associations between the serum vitamin D level and the PA volume or PA intensity for different age and sex groups. The serum vitamin D level was the dependent variable. Sun exposure is the main source of vitamin D in the human body. Two adjusted models were used in the analyses to better illustrate the role of sun exposure in the relationship between PA and serum vitamin D levels. Model 1 was adjusted for race/ethnicity, BMI category, PIR, and total vitamin D intake. Model 2 further incorporated the amount of time spent outdoors time in addition to all the variables in Model 1.

After identifying subgroups in which a significant correlation existed between PA and the serum vitamin D level through a stratified multivariate linear regression analysis across the two models, we proceeded to evaluate the potential mediating effects of BMI and the amount of time spent outdoors on the relationship between PA and the serum vitamin D level within these identified subgroups using a mediation model.

These mediation models were adjusted for race/ethnicity, BMI, PIR, total vitamin D intake, and the amount of time spent outdoors, except when covariates such as BMI or outdoor time were used as mediating variables. The average direct effect represents the effects of PA levels on serum vitamin D levels without a mediator. The average causal mediation effect (ACME) indicates that the influence of PA levels on serum vitamin D levels is mediated through BMI or outdoor time as an intermediary factor. The proportion of mediation was determined by dividing the ACME by the total effect. The proportion mediated was estimated when the mediated effect was significant.

Statistical analyses were performed using R software 4.2.0 (The R Foundation, http://www.R-project.org). The mediation analyses were executed via the "mediation" R package (version 4.5.0). All the statistical tests were conducted with two-sided significance, and a p value less than 0.05 was considered to indicate statistical significance.

Results

Study participants and baseline characteristics

Among the 19,931 original cohort members, 3,857 individuals were aged 3–20 years and had no missing information on vitamin D levels, PA levels or the amount of time spent outdoors. Among these 3,854 individuals, three were excluded for excessive vitamin D intake or excessive time spent outdoors. Additionally, 542 individuals with missing information on the covariates were further excluded. Ultimately, 3,312 eligible participants were included in this study. The selection flow chart is shown in Figure 1.

A total of 3,312 participants were included in the study; 1,672 were boys (50.4%), and 1,640 were girls (49.6%). A total of 250 (7.5%) children were aged 3–5 years, 1,474 (44.5%) were aged 6–11 years, and 1,588 (47.9%) were aged 12–19 years. As shown in Table 1, no significant differences in the sex distributions of the 3–5-year-old group, the 6–11-year-old group, or the 12–19-year-old group were observed ($p > 0.05$).

As shown in Table 1, among the participants, 94 children (2.8%), including 51 boys and 43 girls, had a BMI < 5th percentile, and 1,315 (39.7%) had a BMI ≥ 85th percentile. The intake of vitamin D by girls aged 6–11 and 12–19 years was lower than that of boys in the corresponding age groups.

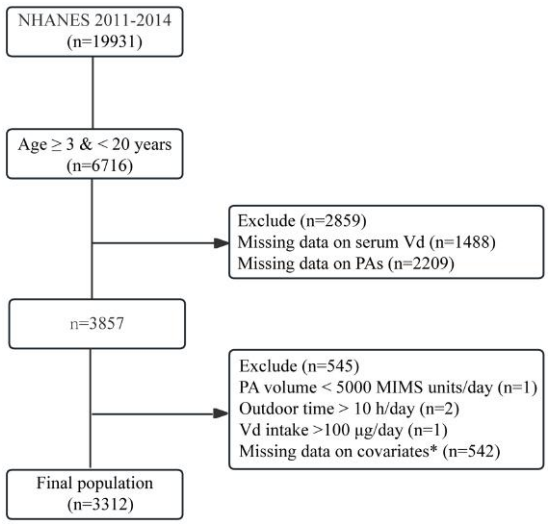

FIGURE 1
Flowchart of participant selection. *Covariates included age, sex, race/ethnicity, body mass index, poverty–income ratio, and Vd intake.

Distribution of vitamin D levels

As shown in Table 1 and Figure 2, for children of the same sex, the serum vitamin D levels in the 12–19-year-old group (males, 57.4 ± 18.7 nmol/L; females, 54.8 ± 21.8 nmol/L) were lower than those in the 6–11-year-old group (males, 65.8 ± 16.5 nmol/L; females, 62.1 ± 18.9 nmol/L), and the 3–5-year-old group (males, 72.4 ± 16.6 nmol/L; females, 69.4 ± 17.6 nmol/L).

The serum vitamin D levels of the girls in the 6–11-year-old group and in the 12–19-year-old group were lower than those of the boys in the corresponding age groups. A statistically significant difference in vitamin D levels was not observed between boys and girls in the 3–5-year-old group.

Distribution of PA volume according to age and sex

As shown in Table 1 and Figure 2, for individuals of the same sex, the PA volume in the 12–19-year-old group was lower than that in the 3–5-year-old group and the 6–11-year-old group for boys or girls ($p < 0.05$). No statistically significant difference in the PA volume was observed between the 3–5-year-old group and the 6–11-year-old group of boys or girls.

TABLE 1 Study participants and baseline characteristics.

Characteristic	3–5-year-old group			6–11-year-old group			12–19-year-old group		
	Male	Female	*p*	Male	Female	*p*	Male	Female	*p*
N (%)	126 (50%)	124 (50%)		749 (51%)	725 (49%)		797 (50%)	791 (50%)	
Race/ethnicity[#]			>0.9			0.3			0.088
Mexican American	35 (28%)	32 (26%)		159 (21%)	175 (24%)		157 (20%)	173 (22%)	
Other Hispanic	15 (12%)	13 (10%)		83 (11%)	63 (8.7%)		75 (9.4%)	91 (12%)	
Non-Hispanic White	25 (20%)	30 (24%)		214 (29%)	185 (26%)		220 (28%)	185 (23%)	
Non-Hispanic Black	27 (21%)	27 (22%)		201 (27%)	206 (28%)		225 (28%)	202 (26%)	
Other/Multiracial	24 (19%)	22 (18%)		92 (12%)	96 (13%)		120 (15%)	140 (18%)	
BMI category[#]			0.3			0.3			0.7
Underweight	4 (3.2%)	10 (8.1%)		22 (2.9%)	12 (1.7%)		25 (3.1%)	21 (2.7%)	
Normal weight	92 (73%)	83 (67%)		416 (56%)	403 (56%)		447 (56%)	462 (58%)	
Overweight	16 (13%)	13 (10%)		128 (17%)	140 (19%)		137 (17%)	137 (17%)	
Obese	14 (11%)	18 (15%)		183 (24%)	170 (23%)		188 (24%)	171 (22%)	
PIR*	1.89 ± 1.48	1.89 ± 1.59	0.6	1.91 ± 1.52	1.87 ± 1.51	0.6	2.11 ± 1.59	1.96 ± 1.53	<0.05
Total vitamin D intake* (μg)	6.3 (3.9, 12.9)	7.0 (3.6, 12.2)	0.9	6.7 (4.4, 10.7)	6.0 (3.6, 9.8)	<0.05	5.4 (2.7, 10.0)	3.8 (2.0, 6.7)	<0.05
Serum vitamin D* (nmol/L)	72.4 ± 16.6	69.4 ± 17.6	0.2	65.8 ± 16.5	62.1 ± 18.9	<0.05	57.4 ± 18.7	54.8 ± 21.8	<0.05
PA volume* (MIMS Units/day)	19,813 ± 2,859	19,127 ± 2,850	0.080	19,435 ± 3,530	19,020 ± 3,082	<0.05	14,270 ± 3,604	14,419 ± 3,189	<0.05
PA intensity* (MIMS units/min)	64.2 ± 10.7	58.9 ± 9.3	<0.05	65.9 ± 12.3	59.7 ± 10.2	<0.05	49.3 ± 11.6	45.8 ± 7.6	<0.05
Outdoor time* (min/day)	95.6 ± 60.0	91.4 ± 61.6	0.5	107.6 ± 66.7	99.0 ± 61.1	<0.05	100.8 ± 67.9	81.1 ± 56.9	<0.05

*Means ± SDs; medians (IQRs); the Wilcoxon rank sum test was applied. [#]Pearson's chi-square test was applied. Outdoor time, the amount of time spent outdoors.

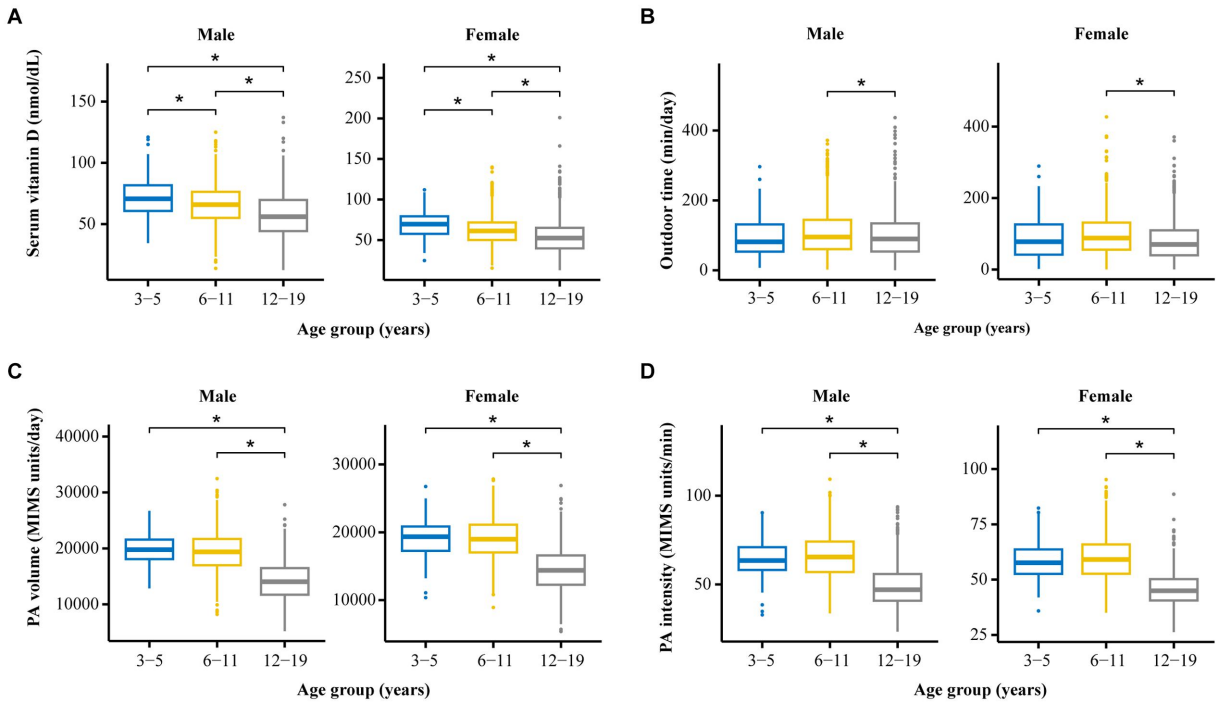

FIGURE 2
The distribution and differences among four crucial variables (vitamin D level, PA volume, PA intensity, and outdoor time) among groups stratified by age and sex. **(A)** Comparison of serum vitamin D levels among participants of different ages and different sexes. **(B)** Comparison of outdoor time levels among participants of different ages and different sexes. **(C)** Comparison of PA volumes among participants of different ages and different sexes. **(D)** Comparison of PA intensity levels among participants of different ages and different sexes. *$p < 0.05$.

Within the same age groups, a statistically significant difference in PA volume was not observed between boys and girls in the 3–5-year-old group or the 12–19-year-old group ($p > 0.05$). The PA volume of boys was greater than that of girls in the 6–11-year-old group ($p < 0.05$).

Distribution of PA intensity levels in children of different sexes and ages

As shown in Table 1 and Figure 2, for children of the same sex, the PA intensity was significantly lower in the 12–19-year-old group than in the 3–5-year-old group and the 6–11-year-old group ($p < 0.01$) of boys or girls. Moreover, a statistically significant difference in PA intensity was not observed between the 3–5-year-old and the 6–11-year-old groups ($p > 0.05$) of boys or girls.

Similarly, the PA intensity of boys in the 3–5-year-old group, the 6–11-year-old group, and the 12–19-year-old group was greater than that of girls in the corresponding age group ($p < 0.05$).

Distribution of the amount of time spent outdoors by children of different sexes and ages

As shown in Table 1 and Figure 2, for children of the same age, the amount of time spent outdoors by girls in the 12–19-year-old group and the 6–11-year-old group was significantly less than that spent by boys in the corresponding group ($p < 0.05$). A statistically significant difference in the amount of time spent outdoors was not observed between males and females in the 3–5-year-old group.

According to the analysis of children of the same sex, girls in the 12–19-year-old group spent less time outdoors than did those in the 3–5-year-old group and the 6–11-year-old group; moreover, no statistically significant difference in this parameter was observed for boys among the three age groups.

Relationships between PA and serum vitamin D levels in different sex and age groups

Relationship between the PA volume and the serum vitamin D level

As shown in Figure 3, after excluding the impacts of the amount of time spent outdoors, race/ethnicity, BMI, PIR, and total vitamin D intake, no correlation was detected between the PA volume and serum vitamin D levels in boys in the 3–5-year-old group. A positive correlation was identified between the PA volume and serum vitamin D levels in both boys and girls in the 6–11-year-old group.

In the 12–19-year-old group, a positive correlation was observed between the PA volume and serum vitamin D levels only in boys in Model 1 and Model 2. In the 12–19-year-old female group, a positive correlation was observed between the PA volume and serum vitamin D levels only in Model 1, which was adjusted for race/ethnicity, BMI, PIR, and total vitamin D intake, and no correlation between the PA volume and serum vitamin D levels was found in Model 2, which was adjusted for all the variables in Model 1 plus the amount of time spent outdoors.

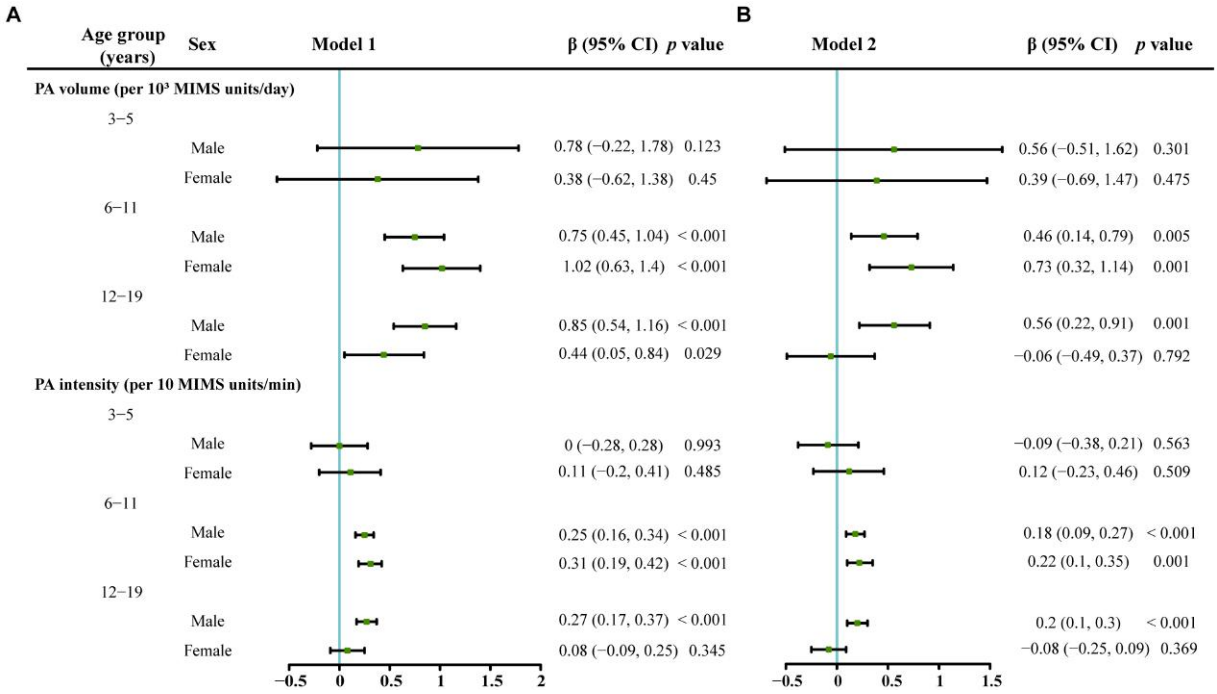

FIGURE 3
The associations between serum vitamin D levels and PA volume or PA intensity stratified by age and gender analyzed using a multivariate linear regression model. The serum vitamin D level was the dependent variable. **(A)** Model 1 which was adjusted for race/ethnicity, the classification of the body mass index, poverty–income ratio and vitamin D intake. **(B)** Model 2 which was additionally adjusted for outdoor time based on Model 1.

Relationship between PA intensity and serum vitamin D levels

As shown in Figure 3, in the 3–5-year-old group, PA intensity was not related to the serum vitamin D level ($p > 0.05$). In the 6–11-year-old group, a positive correlation was identified between the PA intensity and serum vitamin D levels in boys and girls in Model 1 and Model 2 ($p < 0.05$). In the 12–19-year-old group, a positive correlation was observed between the PA intensity and serum vitamin D levels only in boys in Model 1 and Model 2 ($p < 0.05$).

The mediating effects of the amount of time spent outdoors and BMI on the relationship between PA and vitamin D levels

As shown in Table 2, the amount of time spent outdoors had a mediating effect on the relationship between serum vitamin D levels and PA, including the PA volume and PA intensity, in boys aged 12–19 years and in girls and boys aged 6–11 years.

As shown in Table 2, the smallest mediating effect of the amount of time spent outdoors on the relationship between PA and serum vitamin D levels was 28%, and the largest mediating effect of the amount of time spent outdoors on the relationship between PA and serum vitamin D levels was 46%.

Body mass index had a mediating effect on the relationship between serum vitamin D levels and PA, including the PA volume and PA intensity, whereas BMI had no mediating effect on the relationship between the PA volume and serum vitamin D levels only in boys aged 12–19 years. As shown in Table 2, the range of the proportion of the mediating effects of BMI on the relationship between PA and vitamin D levels was 21–34%.

Discussion

The present study showed that the relationship between PA and vitamin D varies among different age and sex groups. We found no correlation between PA, including the PA volume and PA intensity, and serum vitamin D levels in boys and girls aged 3–5 years, but we did observe a positive correlation between the PA volume or PA intensity and vitamin D levels in boys and girls aged 6–11 years and in boys aged 12–19 years. These findings are similar to those of previous studies.

Kyungchul Song et al. studied 3,183 participants aged 12–18 years in the Korea National Health and Nutrition Examination Survey (KNHANES) and reported that individuals with normal vitamin D levels had greater PA levels than individuals with vitamin D deficiency (15). Al Othman et al. (16) conducted a cross-sectional study among 331 children aged 6–17 years (153 boys and 178 girls) in Saudi Arabia and reported that, for an equivalent duration of sunlight exposure, individuals with moderate-to-high physical activity levels presented higher levels of vitamin D. Kim et al. (29) used data from the KNHANES to study the relationship between PA levels and vitamin D levels in adolescents and reported that those who did not participate or who only participated in PA for 1–3 days presented a greater prevalence of vitamin D deficiency than did those who engaged in 4–7 days of activity per week. Our research results are similar to those of the studies described above; however, no relationship was observed between PA and serum vitamin D levels in the groups of girls aged 12–19 years.

TABLE 2 Estimated proportions of the associations between different dependent variables and serum Vitamin D levels mediated by BMI and the amount of time spent outdoors in the subgroups.*

Variable	Sex	Age group (years)	Total effect (95% CI)	Direct effect (95% CI)	Mediated effect (95% CI)	Proportion mediated, %
BMI						
PA volume (per 10^3 MIMS units/day)						
	Male	6–11	0.53 (0.19, 0.84)	0.35 (0.02, 0.68)	0.18 (0.09, 0.27)	33
	Female	6–11	0.77 (0.36, 1.16)	0.61 (0.2, 1)	0.16 (0.08, 0.25)	21
	Male	12–19	0.57 (0.22, 0.9)	0.52 (0.15, 0.85)	0.05 (−0.02, 0.12)	–
PA intensity (per 10 MIMS units/min)						
	Male	6–11	0.21 (0.13, 0.29)	0.15 (0.06, 0.23)	0.06 (0.03, 0.09)	30
	Female	6–11	0.25 (0.14, 0.37)	0.17 (0.04, 0.28)	0.09 (0.05, 0.13)	34
	Male	12–19	0.23 (0.13, 0.33)	0.18 (0.08, 0.28)	0.05 (0.03, 0.08)	22
Outdoor time						
PA volume (per 10^3 MIMS units/day)						
	Male	6–11	0.65 (0.35, 0.94)	0.35 (0.02, 0.68)	0.3 (0.15, 0.45)	46
	Female	6–11	0.91 (0.53, 1.28)	0.61 (0.2, 1)	0.3 (0.15, 0.49)	33
	Male	12–19	0.82 (0.47, 1.14)	0.52 (0.15, 0.85)	0.3 (0.13, 0.48)	36
PA intensity (per 10 MIMS units/min)						
	Male	6–11	0.22 (0.13, 0.3)	0.15 (0.06, 0.23)	0.07 (0.04, 0.11)	33
	Female	6–11	0.26 (0.13, 0.37)	0.17 (0.04, 0.28)	0.09 (0.05, 0.15)	36
	Male	12–19	0.25 (0.15, 0.35)	0.18 (0.08, 0.28)	0.07 (0.03, 0.11)	28

*The model was adjusted for race/ethnicity, BMI, poverty–income ratio, Vitamin D intake and the amount of time spent outdoors, except when the variable was removed due to its role as a mediating variable.

Van den Heuvel et al. (30) evaluated the effects of PA characteristics (such as duration and intensity) on plasma vitamin D levels and reported that high-intensity PA is positively correlated with vitamin D levels. A lower level of PA intensity may be one of the reasons that PA was related to the serum vitamin D level in girls and boys aged 12–19 years in our study.

Our research showed that for children aged 3–5 years, regardless of sex, no relationship existed between the PA duration or intensity and serum vitamin D levels. Similarly, Charlotte Mortensen's (31) study revealed a close correlation between vitamin D levels in 4–8-year-old children and sunlight exposure, whereas PA levels in 4–8-year-old children were not related to vitamin D levels. Moreover, our research revealed no statistically significant differences in the duration or intensity of PA between children aged 3–5 years and those aged 6–11 years. Why is no correlation observed between PA and vitamin D levels in this age group? We did not find any further research on the relationship between PA and vitamin D levels in children aged 3–5 years.

We found a correlation between PA and vitamin D levels in children aged 6–11 years. However, some studies have shown that athletes have a greater prevalence of vitamin D deficiency, which is a very prominent problem (32, 33). In addition, a large-scale meta-analysis of 23 studies involving 2,313 athletes showed that 56% of them were vitamin D deficient (34). Several studies have shown that the vitamin D levels of athletes vary by latitude (35), and athletes who engage in indoor sports have a greater incidence of vitamin D deficiency (32, 36). Moreover, the study by Aydin et al. (37) showed that the difference in vitamin D levels between outdoor and indoor athletes is evident, with 59% of outdoor athletes and 64% of indoor athletes generally experiencing vitamin D deficiency. These studies suggest that the level of vitamin D in athletes is related to the synthesis of vitamin D through sunlight exposure. In our study, through a mediation analysis, we found that sunlight exposure had a mediating effect on the relationship between PA and serum vitamin D levels and that the mediating effect of sunlight exposure in children aged 6–11 years and boys aged 12–19 years ranged from 28% to 46%, indicating that sunlight exposure plays an undeniable role in the relationship between PA and serum vitamin D levels.

What are the mechanisms related to PA and serum vitamin D levels? At present, the underlying mechanisms are unclear. PA can alter the balance of the body, change the levels of circulating media and hormones, and increase the energy demand of skeletal muscles and other important organs. Moreover, PA can promote bone and mineral metabolism, particularly calcium and phosphate metabolism (38), which are crucial for neuromuscular signaling, the biosynthesis of adenosine triphosphate (ATP), and other components of energy metabolism. Additionally, PA alters fat metabolism, which is a site at which inactive vitamin D is stored (39). In our study, we also found that BMI had a mediating effect on the relationship between PA and vitamin D levels. These changes caused by PA may lead to the acceleration of the release and activation of stored inactive vitamin D in the body, thereby increasing vitamin D levels to ensure calcium balance. Athletes may experience vitamin D deficiency due to continuous excessive exercise or insufficient levels of stored vitamin D. In our study, excluding the amount of time spent outdoors, BMI, age, etc., we did not find any statistically significant relationship between PA and serum vitamin D levels in children aged 3–5 years

and girls aged 12–18 years, which may be due to insufficient muscle mass, insufficient PA volume or intensity, or insufficient vitamin D storage. In individuals with these conditions, changes in the calcium level cannot be stimulated by PA through muscle and bone metabolism, thereby preventing the activation and release of inactive vitamin D stored in fat. Further in-depth research is needed on the relationships and possible mechanisms through which PA promotes an increase in vitamin D levels in people of different ages.

A strength of our research is that we found that the relationship between PA and vitamin D levels varied among children and adolescents aged 3–19 years, providing a new perspective for further studies exploring the relationship between PA and vitamin D levels. Second, we found that the amount of time spent outdoors, which mainly means being exposed to sunlight, and BMI have mediating effects on the relationship between PA and vitamin D levels, which will contribute to studies of the relationship and mechanism between PA and vitamin D.

Our study extracted data from the Nutrition Examination Survey (NHANES), which is a cross-sectional survey of United States national health; therefore, the limitation of the present study was that the results cannot reveal a causal relationship between PA and serum vitamin D levels. However, the results of the present cross-sectional study can provide clues for further causal and mechanistic research. Second, the sample sizes of boys and girls aged 3–5 years were relatively small, 126 and 124, respectively, and the 95% confidence intervals of the groups aged 3–5 years were larger; therefore, the relationship between PA and vitamin D levels in children aged 3–5 years needs to be studied further after increasing the sample size.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The studies involving humans were approved by the National Center for Health Statistics Research Ethics Review Board (Protocol #2011-17). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

SO: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. QL: Conceptualization, Writing – review & editing. ZL: Conceptualization, Methodology, Writing – review & editing. YY: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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The complex relationship between vitamin D and kidney stones: balance, risks, and prevention strategies

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The association between vitamin D and kidney stones is characterized by a remarkable multi-dimensional complexity involving numerous physiological and metabolic pathways. Vitamin D is pivotal in maintaining calcium-phosphorus metabolic homeostasis and bone health. However, fluctuations in its intake, whether excessive or insufficient, May potentially increase the risk of kidney stones. Vitamin D exerts its influence on kidney stone formation indirectly by increasing the efficiency of intestinal calcium absorption and regulating renal calcium excretion. Moreover, there is a robust correlation between various states of vitamin D, particularly its active form, 1,25-dihydroxyvitamin D, and the development of numerous kidney stones. This finding underscores the necessity of individualized medical treatment in vitamin D supplementation and kidney stone prevention. When developing treatment strategies, it is essential to consider the patient's genetic background, lifestyle, environmental factors, and overall health. To prevent the formation of kidney stones, it is recommended that patients adopt a comprehensive approach, which May include measures such as moderate sun exposure, dietary modification, moderate exercise, and weight management. These preventive measures are designed to maintain healthy calcium and phosphorus metabolism and reduce kidney stone formation risk. Future studies should aim to elucidate the detailed mechanisms of vitamin D metabolism, individual differences, and the role of genes in this process. Furthermore, the role of lifestyle interventions in preventing kidney stones requires greater attention. Moreover, the implementation of large-scale, long-term prospective studies and randomized controlled trials will facilitate the assessment of the actual effects of diverse vitamin D supplementation strategies, thereby providing a robust scientific foundation for advancing more precise prevention strategies and clinical guidelines.

KEYWORDS

vitamin D, kidney stones, calcium and phosphorus metabolism, personalized medicine, lifestyle intervention

1 Introduction

Vitamin D, a widely recognized nutrient, has recently attracted significant attention from the scientific community and the general public. Its physiological role in the human body is far-reaching and extensive. It is directly involved in calcium and phosphorus metabolism and the maintenance of bone health and plays a vital role in regulating immune function (1, 2).

The biological effects of vitamin D extend beyond those related to bone health, as it has been shown to influence several physiological processes, thereby underscoring its indispensable value. Conversely, kidney stones, a prevalent affliction of the urinary system, are precipitated by many factors, including dietary habits, lifestyle, and genetic predispositions (3–6). Although the precise mechanism of kidney stone formation remains to be elucidated, it is widely accepted that the deposition of calcium, uric acid, and oxalic acid is a primary causative factor (7–9).

An in-depth investigation of the relationship between vitamin D and kidney stones is of critical importance for a comprehensive understanding of the physiologic functions of vitamin D and the pathogenesis of kidney stones. A deficiency in vitamin D has been demonstrated to potentially elevate the likelihood of developing kidney stones (10–12). Consequently, administering an appropriate vitamin D dosage to individuals exhibiting deficiencies May prove a productive method for mitigating the risk of kidney stones. Nevertheless, there is no consensus among the academic community regarding the precise relationship between vitamin D intake and the risk of developing kidney stones. Some studies indicate that vitamin D supplementation May result in elevated blood calcium levels, subsequently increasing urinary calcium excretion and ultimately elevating the risk of kidney stones (13–16). However, other studies have reached the opposite conclusion, indicating that although vitamin D supplementation May result in alterations in calcium metabolism and an increased risk of hypercalcemia and hypercalciuria, it does not increase the risk of kidney stones (17, 18). Some studies have concluded that vitamin D supplementation does not significantly affect serum calcium concentration and urinary calcium excretion (19, 20). It is evident that the relationship between vitamin D and kidney stones is complex and requires further investigation. Consequently, a comprehensive investigation into the relationship between vitamin D and kidney stones is paramount. This helps us understand the physiological function of vitamin D more comprehensively and provides new ideas and methods for preventing and treating kidney stones. This article aims to review the relevant studies conducted in recent years comprehensively. This review is intended to serve as a valuable reference for researchers and clinical practitioners in related fields and facilitate the advancement of academic knowledge and clinical applications in this field.

2 Physiological functions of vitamin D

2.1 Sources and metabolic pathways of vitamin D

Vitamin D is a crucial fat-soluble vitamin that maintains bodily health. It has two primary forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) (21). Vitamin D2 is primarily derived from plant sources, while the skin synthesizes vitamin D3 in response to sunlight. The synthesis of vitamin D3 begins with 7-dehydrocholesterol (7-DHC) in the skin, which is converted to cholecalciferol in the presence of ultraviolet B (UVB) radiation (22). In addition to dermal synthesis, vitamin D can be ingested through food, particularly oily fish (e.g., salmon and mackerel), fortified dairy products, egg yolks, and certain mushrooms high in the vitamin (23–25).

The activation of vitamin D in the body necessitates a two-step conversion process. First, vitamin D is converted to 25-hydroxyvitamin D (25(OH)D), its primary circulating form in the liver (26). Subsequently, in the kidneys, 25(OH)D is further converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active form of vitamin D responsible for regulating calcium and phosphorus metabolism (Figure 1) (27–29).

2.2 Role of vitamin D in calcium and phosphorus metabolism

Vitamin D plays a crucial role in maintaining the body's equilibrium of calcium and phosphorus. It Markedly enhances the bioavailability of these two essential minerals by stimulating the active intestinal absorption processes of calcium and phosphorus (30, 31). Specifically, vitamin D binds to the vitamin D receptor (VDR) in intestinal cells, activating several physiological responses. One significant consequence is an enhancement in the efficacy of calcium transport across the gastrointestinal epithelium via the transient receptor potential vanilloid 6 (TRPV6) transporter protein, which optimizes the intestinal absorption of calcium (32). Vitamin D sufficiency induces intestinal cells to synthesize calcium-binding proteins, which bind specifically to calcium ions, enhancing the intestinal affinity for calcium ions and thus facilitating calcium absorption (33, 34). Furthermore, vitamin D benefits phosphorus absorption, thereby ensuring the effective utilization of phosphorus in the body.

Vitamin D plays a pivotal role in regulating hormone secretion, thereby maintaining the blood's equilibrium of calcium and phosphorus concentrations. This mechanism is primarily achieved through the influence of vitamin D on the secretion of parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) (35, 36). In the metabolic milieu of the kidney, the interaction between vitamin D, FGF23, and PTH is essential for maintaining homeostasis of calcium and phosphorus metabolism (37). Specifically, when vitamin D activity is enhanced, it Decreases the level of PTH. This, in turn, Decreases renal reabsorption of calcium and phosphorus, leading to increased urinary excretion of calcium and phosphorus. Conversely, elevated vitamin D activity stimulates the expression of FGF23, which, by inhibiting the activity of 1α-hydroxylase (CYP27B1) and activating 24-hydroxylase (CYP24A1), a pivotal enzyme in the degradation of vitamin D, in turn reduces the production of active vitamin D ($1,25(OH)_2D$) (38). In contrast, PTH plays a distinct role in maintaining calcium-phosphorus balance. When the concentration of calcium in the bloodstream Declines, the secretion of PTH is initiated, which, in turn, increases the reabsorption of calcium by the kidneys (38). Concurrently, PTH stimulates the production of $1,25(OH)_2D$ in the kidneys by increasing the activity of 1α-hydroxylase and Decreasing the activity of 24-hydroxylase, thereby enhancing intestinal calcium absorption and elevating the calcium concentration in the blood (39). In the presence of elevated blood calcium levels, vitamin D stimulates the production of FGF23, which, by reducing the reabsorption of phosphorus by the kidneys, helps to lower blood phosphorus concentrations (35). This, in turn, prevents the onset of physiological disturbances that high blood calcium levels May trigger. This intricate and precisely calibrated regulatory network ensures the maintenance of calcium and phosphorus metabolism within the body, which is crucial for preserving bone health and sustaining other physiological processes.

The impact of vitamin D on the process of bone remodeling is considerable. Skeletal remodeling is a complex and dynamic process

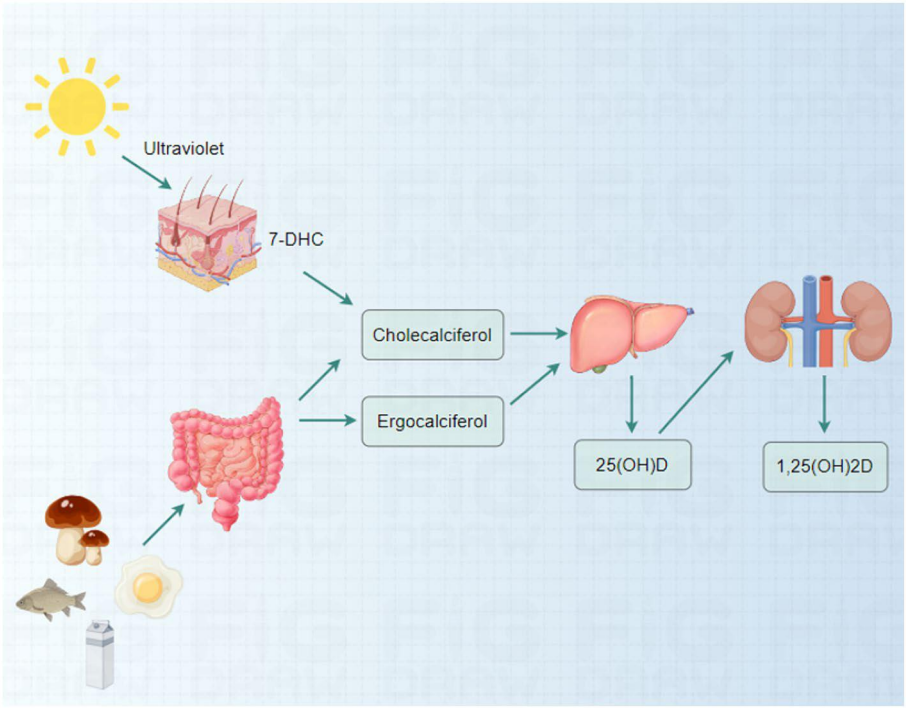

FIGURE 1
Sources and metabolic pathways of vitamin D.

involving a sophisticated interaction between osteoblasts, primarily responsible for bone formation, and osteoclasts, mainly responsible for bone resorption (40). Vitamin D plays a pivotal regulatory role in this process, influencing and optimizing the functional state of both types of cells involved in bone renewal and repair. Vitamin D significantly enhances the activity of osteoblasts, which facilitates the synthesis of bone matrix, thereby reinforcing the bone structure. Concurrently, vitamin D also inhibits osteoclast activity, reducing bone loss and maintaining bone stability and integrity (37, 41–43). This two-way regulation mechanism of vitamin D enables the dynamic balance of the bone remodeling process to be better kept, thus effectively preventing osteoporosis and other bone-related diseases.

2.3 Distribution and function of vitamin D receptors in the urinary system

The VDR is a nuclear receptor widely distributed in several body tissues, including the kidneys, urinary tract, and bladder of the urinary system (44). The expression and activity of the VDR are critical to the health and function of the urinary system.

The VDR plays a pivotal role in the regulation of renal electrolyte homeostasis. The VDR is expressed in renal tubular epithelial cells, and vitamin D is involved in the renal reabsorption and excretion of calcium and phosphorus by binding to it (45, 46). This regulatory mechanism is essential for maintaining calcium-phosphorus balance within the body and preventing disorders of mineral metabolism. Vitamin D affects the acid–base regulatory mechanism of the kidneys through the VDR, thereby assisting in maintaining the acid–base balance of urine (47, 48). This action is of significant importance in preventing urate and other acid deposition within the urinary tract, thereby preventing the formation of kidney stones. Moreover, VDR May play a role in the indirect regulation of renal electrolyte balance, extending beyond calcium and phosphorus, through its influence on the renin-angiotensin-aldosterone system (RAAS) activity (49). Vitamin D plays a regulatory role in the reabsorption of sodium, potassium, and other electrolytes in the kidneys, which in turn affects the production and excretion of urine. Vitamin D can potentially exert protective effects in chronic kidney disease (CKD) patients. The activation of the VDR has been demonstrated to slow the progression of kidney disease, with mechanisms that May be related to anti-inflammatory, anti-fibrotic, and immunomodulatory functions (50–53). The expression of the VDR in bladder tissues May be linked to smooth muscle function and the urinary storage capacity of the bladder (54). However, research in this area has been relatively limited. The immunomodulatory role of vitamin D is also essential in the urinary system, where it May prevent infection and inflammation by modulating local immune responses (12, 55).

3 Relationship between vitamin D and kidney stones

3.1 Mechanism of vitamin D in kidney stone formation

Vitamin D, particularly its active form, $1,25(OH)_2D$, plays a complex and multifaceted role in forming kidney stones. Specifically, vitamin D facilitates intestinal calcium absorption by binding to the VDR on intestinal cells (32, 34, 56, 57). This physiological process May result in elevated blood calcium levels, leading to hypercalcemia. In a

hypercalcemic state, the inhibition of calcium transport May outweigh the inhibition of sodium and calcium absorption by medullary collaterals, ultimately resulting in increased urinary calcium excretion (58). Furthermore, elevated $1,25(OH)_2D$ circulating levels May contribute to an additional increase in urinary calcium excretion (59, 60). Many studies have demonstrated that vitamin D intake increases urinary calcium excretion (18, 61, 62). For patients with hypercalciuria, a population at high risk for stone formation, the increased calcium excretion due to vitamin D intake will further exacerbate their risk of stone formation. This is because patients with hypercalciuria are more susceptible to stone formation. Even minor elevations in urinary calcium can precipitate a notable increase in calcium oxalate supersaturation.

$1,25(OH)_2D$ also regulates the expression of calcium-sensing receptors (CaSR) and calcium-binding proteins in the kidney and other tissues (45, 63–65). These proteins play a pivotal role in intracellular calcium transport and excretion. The CaSR can effectively inhibit paracellular calcium transport by up-regulating claudin-14 expression, reducing renal tubular permeability to calcium ions and thus maintaining stable intracellular calcium concentrations (64, 65). Transient receptor potential cation channel subfamily C, member 3 (TRPC3) is expressed in the proximal tubule (PT) and plays a role in the transcellular calcium reabsorption process at this site through CaSR activation (66). It is noteworthy that TRPC3 knockout mice exhibited hypercalciuria and microcalcification, demonstrating the protective role of TRPC3 in preventing kidney stone formation (66). Furthermore, proximal tubule cells are particularly susceptible to oxidative damage from excess reactive oxygen species (ROS) (67, 68). In a mouse model of TRPC3 ablation, increased hypercalciuria resulted in heightened oxidative stress, precipitating PT cell injury and ultimately forming mixed stones. This finding further confirms the critical role of TRPC3 in the occurrence and development of kidney stones (69). Furthermore, it has been demonstrated that TRPC3-like proteins are involved in the vital process of capacitative cation entry induced by 1α,25-dihydroxyvitamin D3 under conditions of ROS (70). Consequently, vitamin D deficiency or excess May disrupt the typical expression of these pivotal proteins, resulting in an imbalance in calcium reabsorption and excretion and, ultimately, an elevated risk of kidney stone formation.

The etiology of kidney stones is complex, with calcium oxalate stones accounting for more than 80% of cases (71). The formation of calcium oxalate stones is influenced by a combination of factors, with hypercalciuria and hyperoxaluria representing two major risk factors significantly elevating the risk of stones. In-depth studies have revealed that oxalate secretion in renal tubules is mediated through the SLC26A6 protein in the solute linkage carrier 26 gene family (72). Notably, studies in mouse models have demonstrated a significant increase in urinary oxalate concentration and stone formation rate in the presence of upregulated SLC26A6 expression (73). Vitamin D is pivotal in oxalate metabolism and contributes to kidney stone formation. The equilibrium between the absorption and excretion of oxalate, the primary constituent of calcium stones, is paramount in preventing stone formation (74). It has been demonstrated that vitamin D supplementation May accelerate the formation of Randall's plaque, which represents the initiating step in calcium oxalate stone formation (75). Furthermore, vitamin D supplementation May enhance oxalate absorption in the gut, which can result in the development or worsening of hyperoxaluria, thereby increasing the relative saturation of calcium oxalate (76, 77). Both of these conditions can potentially promote the formation of oxalate stones.

Vitamin D plays a pivotal role in regulating calcium deposition in renal tissues. This is achieved primarily through the modulation of the activity of renal calcification inhibitory factors, including receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) (78, 79). The equilibrium of these factors is paramount for the average deposition of calcium salts in the kidney. When imbalanced, it May result in the abnormal deposition of calcium salts in the kidney, which induces stone formation. Furthermore, vitamin D is implicated in the renal acid–base regulatory mechanism, which modulates the solubility of calcium and oxalate by altering the pH of the urine. Changes in urinary pH significantly impact stone formation, directly affecting the solubility of these minerals in the urine (80). Notably, pathological processes such as inflammation, oxidative stress, and angiogenesis have also been strongly associated with kidney stone formation. Vitamin D's anti-inflammatory, antioxidant, and anti-angiogenic properties are essential. These bioactivities mitigate the damage to renal tissue caused by inflammation and oxidative stress and May also play a preventive role by directly intervening in the stone formation process (Figure 2) (12, 81).

Hypercalciuria and calcium kidney stones represent significant risk factors for CKD. The regulatory role of vitamin D in these pathological processes is highly complex. While the exact mechanism has not yet been fully elucidated, it is known to prevent the development of hypercalciuria and calcium kidney stones primarily by regulating blood calcium levels and influencing urinary calcium excretion. Reducing blood calcium levels increases PTH secretion, stimulating the kidneys to produce more significant quantities of $1,25(OH)_2D$. This response facilitates the absorption of calcium in the intestines and the release of calcium from bones, thereby maintaining stable blood calcium levels. Conversely, vitamin D metabolism is inhibited when blood calcium levels are elevated, reducing calcium absorption and preventing hypercalcemia. It is important to note that although vitamin D does not directly regulate urinary calcium excretion, it indirectly affects this process by influencing PTH secretion and renal VDR activity. In typical circumstances, vitamin D and PTH function in concert to ensure the equilibrium of calcium levels in the blood and urine. It is also important to note that prolonged excessive vitamin D intake May result in hypercalcemia and hypercalciuria. Consequently, the regulation of vitamin D requires particular caution for patients presenting with hypercalciuria and calcium kidney stones. It is crucial to maintain reasonable control of vitamin D intake and to conduct close monitoring of blood and urine calcium levels to prevent the development of CKD.

The role of vitamin D becomes particularly complex and essential in the context of patients with CKD. CKD is often accompanied by disturbances in vitamin D metabolism, and vitamin D deficiency is commonly observed in CKD and its disease progression. A comprehensive analysis of 367 pre-dialysis patients revealed that over 80% exhibited vitamin D deficiency. This deficiency reflects the significant impact of Decreased renal function on the anabolic and catabolic phases of vitamin D metabolism. Further, it disturbs the calcium and phosphorus homeostasis, significantly increasing the risk of kidney stone formation (82).

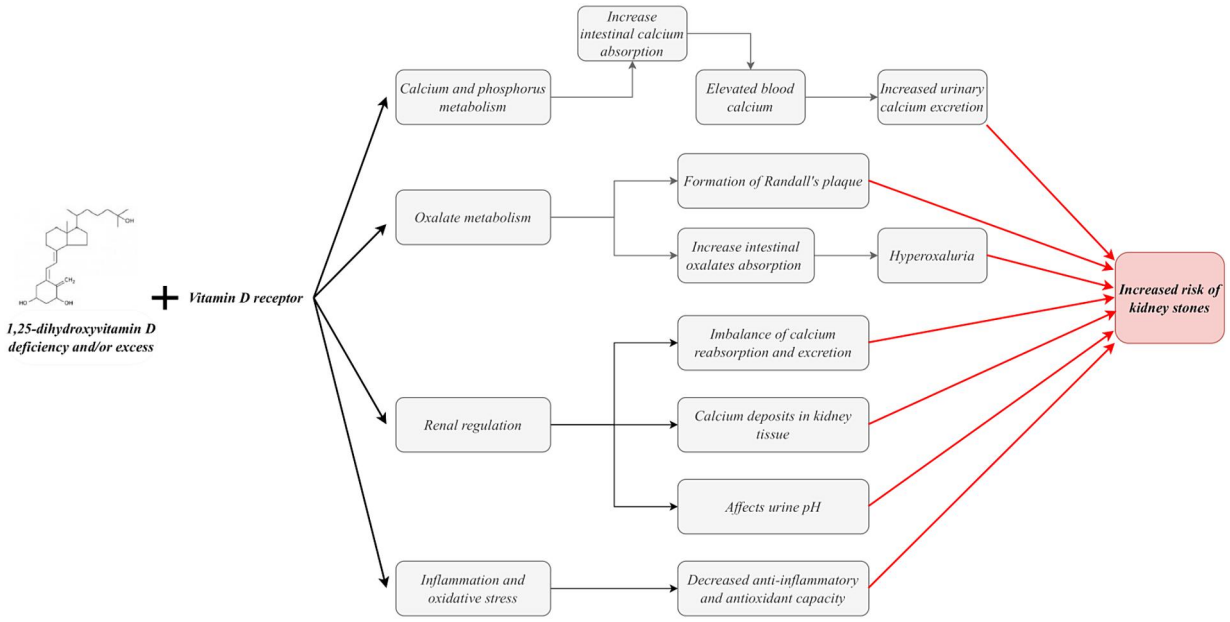

FIGURE 2
Mechanism of vitamin D in kidney stone formation.

3.2 Potential link between vitamin D deficiency and kidney stones

Vitamin D deficiency is typically diagnosed when the concentration of 25(OH)D is less than 20 ng/mL (44, 83, 84). A literature review reveals a significantly higher prevalence of vitamin D deficiency in individuals with urolithiasis than those without (11). Vitamin D deficiency May induce secondary hyperparathyroidism, a compensatory physiological response by the body to maintain homeostasis of blood calcium levels. This is achieved specifically through increased secretion of PTH (12, 83). Elevated levels of PTH further promote the release of calcium from bone into the bloodstream and inhibit renal reabsorption of calcium, leading to increased urinary calcium excretion. These physiologic changes May elevate the risk of specific types of kidney stones (85). Furthermore, vitamin D deficiency May exacerbate the risk of kidney stone formation by inducing oxidative stress and overexpression of inflammatory mediators in renal tissue (12). The impact of vitamin D deficiency on renal reabsorption function, encompassing the reabsorption of calcium and phosphorus, should also be considered. Despite the lack of complete elucidation, the potential association between vitamin D deficiency and renal function with renal stone formation merits further comprehensive investigation.

3.3 Correlation between vitamin D supplementation and the risk of kidney stones

Vitamin D is pivotal in maintaining bone health and regulating calcium and phosphorus metabolism. The normal range for vitamin D is generally considered 20–50 ng/mL (50–125 nmol/L) (86). However, levels above 100 ng/mL May indicate an overdose, while levels above 150 ng/mL are indicative of vitamin D toxicity. At present, there is no standardized dose of vitamin D supplementation. The usual recommended daily supplementation dose is 400 to 800 international units (IU) (87). However, daily supplementation of approximately 2,000 IU of vitamin D May be necessary to achieve serum 25(OH)D concentrations of ≥30 ng/mL in most of the population (88). The tolerable maximum daily vitamin D intake is typically 4,000 IU/day. It is important to note that supplementation of more than 10,000 IU of vitamin D daily May have toxic effects on the general population (89). The relationship between vitamin D supplementation dose and vitamin D concentration is not linear and is influenced by various factors, including age, gender, health status, and sun exposure.

Academic researchers have no consensus regarding the relationship between vitamin D supplementation and kidney stone risk. Some studies have substantiated the correlation between vitamin D supplementation and an increased risk of kidney stones. In humans and rats, vitamin D supplementation has been demonstrated to result in the development of hypercalciuria, renal calcification, and/or kidney stone formation (17, 90). Several meta-analyses have indicated that vitamin D supplementation increases the risk of kidney stones (13–15). A 3-year, double-blind, randomized controlled trial demonstrated that in healthy adults, vitamin D3 supplementation (400, 4,000, or 10,000 IU per day) resulted in hypercalciuria in 23% of participants, with a higher prevalence observed in the high-dose group (91). Another study of patients with recurrent calcium kidney stones and concomitant vitamin D deficiency revealed that serum 25(OH)D and 24-h urinary calcium levels exhibited a Marked elevation following 8–12 weeks of treatment with 50,000 IU of vitamin D per week (18). In contrast, a meta-analysis indicated that prolonged (≥24 weeks) vitamin D supplementation was associated with an elevated risk of hypercalcemia and hypercalciuria, with no discernible correlation between dosage and risk (17). A longitudinal prospective study also observed that total vitamin D intake was associated with an

elevated risk of kidney stones in a cohort of female nurses aged 25–42 (92). Similarly, supplemental vitamin D intake yielded comparable outcomes (92). A randomized clinical trial conducted by the Women's Health Initiative demonstrated that postmenopausal women who received daily oral vitamin D3 (400 IU) plus calcium (1,000 mg) supplementation exhibited a 17% higher incidence of urinary tract stones compared to the placebo group after a 7-year follow-up period (93, 94). Furthermore, a 4-year, double-blind, placebo-controlled, randomized clinical trial revealed that in healthy postmenopausal women aged 55 years or older, the incidence of both kidney stones and elevated serum calcium levels was significantly higher in the treatment group (receiving vitamin D3 2000 IU/day and calcium 1,500 mg/day) than in the placebo group (95). A Mendelian randomization study also corroborated the correlation between chronically elevated circulating 25-hydroxyvitamin D levels and elevated blood calcium levels, which were associated with an increased risk of kidney stones (16). Vitamin D toxicity can result in hypercalciuria and the formation of kidney stones (96). Polymorphisms in the VDR gene have been demonstrated to affect calcium metabolism, which has been identified as a trigger for the formation of urinary stones (97–99). In patients with recurrent renal stones, vitamin D3 is regarded as a pivotal hormone in the pathogenesis, which May elevate the risk of kidney stones by Augmenting urinary excretion of calcium and phosphorus (60). Vitamin D administration has demonstrated a favorable impact on mitigating the severity of COVID-19, thereby warranting its recommendation as an adjunctive therapeutic option for managing the disease (100, 101). However, it is imperative to consider the potential risk of hypercalciuria and the development of renal stones when utilizing vitamin D in treating patients with COVID-19 (102).

Nevertheless, some studies adopt an alternative perspective. The findings of these studies indicate that, although vitamin D supplementation results in alterations in calcium metabolism and an increased risk of hypercalcemia and hypercalciuria, it does not consequently elevate the risk of kidney stones (17, 18). Other studies have demonstrated that vitamin D supplementation does not impact serum calcium concentrations or urinary calcium excretion (19, 20, 103). For instance, an analysis of individuals with vitamin D deficiency and stone formation demonstrated that 50,000 IU of vitamin D for 8 weeks did not result in elevated urinary calcium levels (103). Another randomized clinical trial examined the effects of low- and high-dose vitamin D supplementation (receiving 1,000 IU of vitamin D per day or 50,000 IU of vitamin D per week for 6 weeks) in vitamin D-deficient stone formers. The results demonstrated that vitamin D supplementation did not increase urinary calcium excretion or calcium oxalate supersaturation (20). Furthermore, a randomized, placebo-controlled, double-blind clinical trial showed that high-dose vitamin D therapy (100,000 IU/month) did not elevate the risk of stone formation or hypercalcemia in the general population (19).

The available literature indicates that moderate supplementation with vitamin D does not typically result in the formation of kidney stones. Vitamin D supplements within the recommended dosage range (less than 4,000 IU per day) do not generally result in imbalances in calcium metabolism or an increased risk of kidney stones. However, it is possible that long-term use of vitamin D May increase the risk of kidney stones, especially when combined with calcium supplementation. This is because excessive vitamin D intake can stimulate an excessive absorption of calcium from the intestines, resulting in elevated calcium concentrations in the blood. When the calcium concentration in the blood is elevated, the excess calcium is excreted in the urine, thereby increasing the calcium concentration in the urine. Furthermore, high-calcium urine is a significant contributing factor to kidney stone formation. When stone-forming substances such as oxalic acid or phosphate are also elevated in the urine, the likelihood of forming calcium oxalate or calcium phosphate stones is increased.

4 Prevention and treatment strategies

4.1 Kidney stone prophylaxis for vitamin D status

Maintaining a healthy vitamin D status is essential to prevent kidney stone formation. Sun exposure is an effective method for the natural synthesis of vitamin D3. It is recommended that individuals engage in moderate sun exposure during the hours of the day when the sun is at its highest point in the sky to facilitate the synthesis of vitamin D within the body. Concurrently, the diet should include foods rich in vitamin D, such as oily fish, cod liver oil, fortified dairy products, and egg yolks (104–106). Furthermore, ensuring an adequate calcium intake can also help reduce oxalate absorption, reducing the risk of calcium oxalate stone formation.

Vitamin D supplements May be considered viable for individuals who cannot obtain adequate amounts of vitamin D through natural sunlight exposure or daily dietary intake. Previous studies have demonstrated that a weekly intake of 30,000 IU of vitamin D supplementation is an effective means of assisting individuals with vitamin D deficiency to achieve standardized vitamin D levels (>30 ng/mL). Further studies have demonstrated that administering the same vitamin D supplements twice weekly for 5 weeks represents a rapid, productive, and secure treatment option for vitamin D deficiency (107). It is crucial to acknowledge that obese patients typically require a dosage of vitamin D supplementation that is two to three times greater than that required by individuals with average body weight when treating vitamin D deficiency (108). This is because obesity May result in a reduction in the bioavailability of vitamin D within the body. Physicians should adhere to clinical guidelines based on the latest research evidence when considering vitamin D supplements for patients with kidney stones (83). Several factors must be considered when making this Decision, including the patient's vitamin D status, the chemical composition of the stones, kidney function, and overall health. While supplementation is necessary for patients with vitamin D deficiency, it is essential to avoid excessive intake to minimize the risk of hypercalciuria and kidney stones.

Vitamin D supplements May be necessary in certain specific situations, such as in patients with osteoporosis, as they help improve bone density and strength (109). Nevertheless, in patients with a history of kidney stones, the use of vitamin D supplements necessitates a more cautious approach and is accompanied by a rigorous risk/benefit assessment (77). In such cases, lower effective doses are recommended, and patients are advised that other precautions, such as increased water intake to promote the excretion of calcium and other salts, will not affect the incidence of incident kidney stones or hypercalcemia (19).

It is paramount that patients with a history of kidney stones who are using vitamin D supplements undergo regular monitoring of serum calcium and 25(OH)D levels, as well as urinary calcium excretion (77). These monitoring results can assist physicians in evaluating the patient's response to vitamin D supplements and facilitate timely dosage adjustments to prevent potential complications. Serum 25(OH)D is a commonly utilized biomarker for assessing vitamin D status within the body. Regular monitoring of serum 25(OH)D levels is essential to ensure that vitamin D status remains within a healthy range. The normal range is generally considered to be 20–50 ng/mL (50–125 nmol/L), while levels above 100 ng/mL May indicate an overdose (83, 86). If a patient's 25(OH)D level exceeds the desired range or urinary calcium excretion is elevated, it May be necessary to reduce the dose of vitamin D (Table 1).

4.2 The role of lifestyle interventions in the prevention of kidney stones

The formation of kidney stones is a complex process influenced by multiple factors, with lifestyle habits playing a significant role. It is possible to significantly reduce the risk of kidney stones by modifying one's lifestyle habits. Diet is an essential factor in the formation of kidney stones. Reducing salt intake reduces urinary calcium excretion, reducing the risk of specific kidney stones (110, 111). A diet high in protein, particularly animal protein, increases the production of uric acid and May result in the acidification of the urine, which contributes to stone formation (112–114). Reducing the consumption of red meat, poultry, and fish, accompanied by an increase in the intake of plant-based proteins, May reduce the risk of uric acid stones (115–117). Conversely, increased fruit and vegetable intake can provide a plentiful supply of potassium and magnesium. These minerals assist in reducing urinary calcium concentrations and lowering the risk of stone formation (118). Supplementing the standard diet with fresh lemon juice May prevent stone recurrence in patients with calcium oxalate kidney stones (119). Furthermore, the moderate consumption of foods rich in calcium (e.g., low-fat dairy products) May reduce the intestinal absorption of oxalates, thereby reducing the risk of calcium oxalate stones (110).

Increasing water intake is one of the most straightforward and productive methods for preventing kidney stones. It plays a pivotal role in preventing the formation of first-time kidney stones and reducing the risk of stone recurrence (118, 120–122). Adequate water intake dilutes stone-forming substances in the urine, reducing the likelihood of their deposition and crystallization. It is advised that fluid intake be increased to at least 2.5 liters per day to prevent the formation of stones (123–125). Furthermore, regular physical activity not only assists in maintaining a healthy weight but also contributes to calcium absorption and bone health by enhancing blood circulation and metabolism and increasing the body's efficiency in utilizing vitamin D (126).

It is well-established that obesity represents a significant risk factor for the formation of kidney stones (127). Excessive adipose tissue is associated with increased uric acid production, increasing the risk of uric acid stones. Furthermore, obese individuals are more likely to engage in limited outdoor physical activity to avoid exposure to sunlight. They are more likely to consume diets low in vitamin D, which can result in vitamin D deficiency (128). Reducing the risk of kidney stones can be achieved by implementing a healthy diet and moderate exercise. Furthermore, weight management can also help improve insulin resistance and other metabolic abnormalities that influence kidney stone formation (118, 129).

While vitamin C is essential for health, excessive intake (primarily through supplements) May increase oxalic acid production, which increases the risk of oxalate stones (130). Both smoking and excessive alcohol consumption are associated with an increased risk of kidney stones (131, 132). Quitting smoking and limiting alcohol intake May improve overall health and reduce the risk of kidney stones (Table 2).

TABLE 1 Vitamin D status and kidney stone prophylaxis.

Vitamin D status	Preventive measures
Deficiency (<20 ng/mL)	1. Increase sun exposure 2. Increase intake of vitamin D-rich foods 3. Vitamin D supplements 4. Monitor serum 25(OH)D levels regularly 5. Monitor 24,25$(OH)_2$D and 1,25$(OH)_2$D, if necessary
Moderate (20–100 ng/mL)	1. Maintain moderate sun exposure and dietary intake 2. Avoid over-supplementation 3. Regular health checkups
Excessive (>100 ng/mL)	1. Reduce use of vitamin D supplements 2. Increase water intake to reduce urinary calcium concentration 3. Closely monitor serum and urinary calcium levels

4.3 Importance of personalized medicine in vitamin D supplementation and kidney stone prevention

The concept of personalized medicine is predicated on tailoring treatment to the patient's genetic background, lifestyle, and environmental factors. Genetics, lifestyle, and environmental factors significantly influence the individual's need for and ability to metabolize vitamin D. Genetic factors, such as polymorphisms in the VDR gene, May affect the synthesis, distribution, and action of vitamin D. Furthermore, lifestyle factors, including diet, physical activity, and sun exposure, May also influence vitamin D status. Environmental factors, such as season and latitude, determine the intensity and duration of sunlight, which in turn affects the skin's ability to synthesize vitamin D. These factors must be considered together when developing a vitamin D supplementation strategy to ensure individualized dosage and form. Furthermore, consideration should be given to the impact of hormones and pharmaceutical agents on vitamin D levels. Although serum 25(OH)D levels are relatively stable, they can be affected by various factors. These factors include thyroid hormones, anticonvulsants, choline, and orlistat. Furthermore, PTH and PTH-related peptides, prolactin, estradiol, testosterone, prostaglandins, and bisphosphonates, in addition to serum calcium and phosphorus, have been demonstrated to induce elevated serum 1,25$(OH)_2$D levels. Conversely, corticosteroids, phospholipases,

TABLE 2 Lifestyle interventions and kidney stone prevention.

Interventions	Preventive effects
Reducing salt intake	Reducing urinary calcium excretion and reducing stone risk
Control high protein diet	Reduce uric acid production to avoid acidification of urine and reduce stone risk
Increase intake of fruits and vegetables	Provide potassium and magnesium to reduce urinary calcium concentration and reduce stone risk
Increase calcium food intake	Decrease intestinal oxalate absorption, reduce calcium oxalate stone risk
Increase water intake	Dilute urine to reduce the chance of stone deposition and crystallization
Regular physical activity	Promote blood circulation and increase the efficiency of vitamin D utilization
Weight management	Improve insulin resistance, reduce uric acid production, reduce stone risk
Control vitamin C, smoking, and alcohol consumption	Avoid excessive vitamin C intake, stop smoking, and limit alcohol consumption to reduce stone risk

ketoconazole, heparin, and thiazides have been shown to reduce serum $1,25(OH)_2D$ levels.

The patient's overall health status and disease risk are crucial considerations when developing a prevention and treatment plan. For instance, patients with kidney stones May be advised to avoid high-dose vitamin D supplementation to reduce the risk of hypercalciuria. Conversely, patients at risk for osteoporosis May require a more aggressive vitamin D supplementation strategy to maintain bone health. Furthermore, the patient's renal function, cardiovascular health, and metabolic status should be considered, as vitamin D metabolism occurs primarily in the liver and kidneys.

5 Limitations of current research and future research directions

5.1 Limitations of the current study

The current body of research on the relationship between vitamin D and kidney stones exhibits considerable inconsistency in its findings. This is evidenced by diverse and even contradictory results at times. This discrepancy May be attributed to variations in study design, sample selection, and experimental methodologies. For instance, some studies have indicated no association between vitamin D intake and the risk of kidney stones (17, 19, 92). In contrast, others have observed that excessive vitamin D intake May increase the risk of kidney stones (13, 95, 133). This inconsistency May also be related to the study populations' genetic background, lifestyle, dietary habits, and dosage and form of vitamin D supplements. It is essential to control for confounding factors to establish causality. However, the complexity of the relationship between lifestyle and environmental factors and vitamin D status and kidney stone risk makes accurate measurement and control of these factors challenging.

The current study is somewhat limited in its scope. Previous studies have primarily focused on the correlation between vitamin D intake and the risk of kidney stones. However, relatively few studies have been conducted on the specific mechanisms of vitamin D metabolism and the differences in the effects of different forms of vitamin D (e.g., D2, D3) on kidney stones. Furthermore, the role of VDR gene polymorphisms in kidney stone formation and the variability among populations of different races and geographic regions have been understudied.

The limitations of sample size and duration of follow-up warrant consideration. Some studies' relatively small sample sizes May not comprehensively represent the entire population. Additionally, the potential for bias in sample selection May result in inaccurate results. Additionally, the brief follow-up duration of certain studies May not permit an accurate evaluation of the long-term effects of long-term vitamin D intake on kidney stone risk. Longitudinal studies with extended follow-up periods are essential for understanding the dynamic relationship between vitamin D status and kidney stone formation.

The need for greater clarity regarding the dose–response relationship is evident. There is a lack of clarity regarding the dose–response relationship between vitamin D intake and the risk of kidney stones. The discrepancy between study results May be attributed to the differing vitamin D intake thresholds employed by different studies to define deficiency or excess. Additionally, no intervention studies have been conducted to assess the impact of adjusting vitamin D intake levels on preventing kidney stones. Such studies are crucial for developing effective prevention strategies and clinical guidelines. Furthermore, randomized controlled trials (RCTs) represent the gold standard for assessing the effectiveness of interventions. However, there May be ethical and practical challenges to implementing RCTs in studies of the effects of vitamin D supplementation on kidney stone risk.

5.2 Directions for future research

A comprehensive examination of the metabolic processes associated with vitamin D and their role in forming kidney stones. Future studies should aim to understand better the metabolic pathways of vitamin D in the body. In particular, they should investigate the conversion of vitamin D to its active form, $1,25(OH)_2D$, and its potential role in kidney stone formation. This process May affect intestinal calcium absorption and renal calcium excretion, which could contribute to the formation of kidney stones. Furthermore, research should concentrate on genetic variation in vitamin D metabolizing enzymes, such as polymorphisms in CYP27B1 and CYP24A1, critical enzymes in vitamin D metabolism. Determining how these polymorphisms affect an individual's vitamin D metabolism and susceptibility to kidney stones is necessary.

Longitudinal studies with a large sample size and a long follow-up period are necessary to understand the relationship between vitamin D and kidney stones comprehensively. It is recommended that large-scale, long-term, population-based prospective studies be conducted to assess the long-term effects of different doses and forms of vitamin D intake on kidney stone risk. To gain a more comprehensive understanding of the relationship between vitamin D intake and

kidney stone risk, these studies must include populations of different ages, sexes, ethnicities, and geographic locations. This will enable the development of more tailored recommendations for other populations. Despite the implementation challenges, future studies should consider conducting randomized controlled trials to assess the effectiveness of different vitamin D supplementation strategies in preventing kidney stones.

When studying the relationship between vitamin D metabolism and kidney stone risk, it is essential to consider the impact of individual differences and genetic factors. Future studies should examine the influence of an individual's genetic background on this relationship. This encompasses the investigation of VDR gene polymorphisms and other genetic variants associated with calcium and phosphorus metabolism. These studies could lead to a more comprehensive understanding of the factors contributing to the higher risk of kidney stones observed in specific populations despite similar levels of vitamin D intake. This understanding could then inform the development of more targeted prevention strategies for high-risk individuals.

An integrated assessment of the combined effects of dietary and lifestyle factors is required. Future studies should integrate the impact of dietary patterns, nutritional intake, lifestyle factors such as physical activity and water intake, and environmental factors such as climate and altitude on vitamin D status and kidney stone risk. This multifactorial approach will facilitate the elucidation of how vitamin D intake and other lifestyle factors collectively contribute to the formation of kidney stones.

It is recommended that interdisciplinary collaboration and international collaborative research be employed. It is recommended that multidisciplinary collaboration, including experts in endocrinology, nutrition, nephrology, genetics, and epidemiology, be encouraged to facilitate a more comprehensive understanding of the relationship between vitamin D and kidney stones. Given the considerable diversity in genetic predispositions, dietary habits, and living environments among populations in different regions, international collaborative studies can facilitate the identification of generalizations and specificities in the vitamin D-kidney stone relationship across diverse populations. Future studies aim to assess the economic impact of different vitamin D supplementation strategies and examine how effective health policies can be developed to prevent vitamin D deficiency and overdose and reduce the incidence of kidney stones.

6 Conclusion

The relationship between vitamin D and kidney stones is intricate and multifaceted, encompassing a multitude of physiologic and metabolic pathways. Both excess and deficiency of vitamin D May increase the risk of kidney stones, necessitating the careful balancing of vitamin D intake. The relationship between vitamin D status and kidney stone risk varies across age, gender, and ethnicity, indicating the need for individualized assessment and management strategies to tailor vitamin D supplementation to the patient's specific situation. Lifestyle modifications, such as dietary changes and increased physical activity, play a significant role in maintaining optimal vitamin D status and preventing the formation of kidney stones.

Future research is necessary to ascertain the causal relationship between vitamin D status and kidney stones, to evaluate the efficacy of various interventions, and to develop individualized treatment regimens. Further studies are required to ascertain the vitamin D requirements and kidney stone risk in specific patient populations. The results of the studies are likely to have significant social and economic implications, including the development of effective prevention strategies and the potential for healthcare cost savings. It is of the utmost importance that interdisciplinary collaboration be fostered and that the translation of research results into practical clinical and public health strategies be pursued with the utmost urgency.

Author contributions

FZ: Conceptualization, Funding acquisition, Project administration, Writing – original draft. WL: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

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Vitamin D deficiency in non-scarring and scarring alopecias: a systematic review and meta-analysis

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Background: Numerous studies have linked vitamin D deficiency (VDD) to the pathogenesis of various alopecia disorders.

Objective: This study aimed to investigate whether patients with alopecia are more likely to have VDD or lower vitamin D levels than controls, and the prevalence of VDD among patients with certain alopecia disorders.

Methods: Electronic searches were conducted using PubMed, Embase, Scopus, and Cochrane Library databases from the dates of their inception until September 2024. Studies that reported data allowing for the calculation of odds ratios, mean differences, or correlation coefficients related to vitamin D levels and alopecia were included, while studies without a confirmed diagnosis of alopecia or those involving patients taking vitamin D supplements were excluded.

Results: It was found that 51.94% of patients with alopecia areata (AA), 50.38% of patients with female pattern hair loss (FPHL), 47.38% of patients with male androgenic alopecia (MAGA), 53.51% of patients with telogen effluvium (TE), and 38.85% of patients with primary scarring alopecia had VDD. Compared to controls, AA patients had a pooled odds ratio (OR) of VDD of 2.84 (95% confidence interval: 1.89–4.26, I^2 = 84.29%, p < 0.01) and a pooled unstandardized mean difference (UMD) of vitamin D levels of −8.20 (−10.28 – −6.12, I^2 = 74.25%, p < 0.01) ng/mL. For FPHL patients, a pooled OR of VDD of 5.24 (1.50–18.33, I^2 = 81.65%, p < 0.01) and a pooled UMD of vitamin D levels of −15.67 (−24.55 – −6.79, I^2 = 91.60%, p < 0.01) ng/mL were found. However, for MAGA, a pooled VDD OR of 4.42 (0.53–36.61, I^2 = 88.40%, p < 0.01), and a pooled UMD of vitamin D levels of −2.19 ng/mL (−4.07 – −0.31 ng/mL, I^2 = 7.64%, p = 0.37) were found. For TE patients, pooled UMD of vitamin D levels of −5.71 (−10.10 – −1.32) ng/mL were found.

Conclusion: People with alopecia frequently have VDD; however, only in patients with AA or FPHL was the association of VDD and decreased vitamin D levels statistically significant compared to control. The findings indicate screening for vitamin D could benefit patients with AA or FPHL, potentially addressing vitamin D deficiency. Further study on vitamin D supplementation as a treatment for alopecia is recommended.

KEYWORDS

vitamin D insufficiency, vitamin D level, hair loss, non-cicatricial alopecia, cicatricial alopecia

1 Introduction

Vitamin D is a lipophilic hormone widely recognized as essential for bone development and calcium homeostasis, and it exerts its effect through the nuclear hormone receptor vitamin D receptor (VDR). Vitamin D is produced in the skin when exposed to sunlight and can also be obtained through diet. The liver synthesizes the primary form of vitamin D, 25-hydroxyvitamin D or 25(OH)D_3, which is then activated in the kidneys by 1a-hydroxylase to produce its biologically active form, 1,25-dihydroxyvitamin D or 1,25$(OH)_2D_3$ (1, 2).

VDR is expressed by T and B lymphocytes, dendritic cells, and macrophages, and 1,25$(OH)_2D_3$ is known to modulate both innate and adaptive immune systems (3). Vitamin D deficiency (VDD) is believed to be an environmental trigger for the onset of autoimmunity, and many studies have found a link between VDD and autoimmune diseases (2–4). The VDR plays a crucial role in hair follicle cycling by regulating hair growth phases, particularly the transition from the anagen phase to the catagen phase (5). Additionally, VDR modulates the immune response in alopecia by interacting with key immune cells, such as T and B lymphocytes, macrophages, and dendritic cells, which are involved in the pathogenesis of autoimmune disorders (6). Non-immune-mediated alopecias (e.g., androgenetic alopecia [AGA] and telogen effluvium [TE]) and immune-mediated hair disorders (e.g., alopecia areata [AA] and primary cicatricial alopecia [PCA] such as frontal fibrosing alopecia [FFA], central centrifugal cicatricial alopecia [CCCA], and lichen planopilaris [LPP]) may therefore be associated with VDD (7, 8).

Alopecia can be classified into non-scarring and scarring types. Non-scarring alopecias are characterized by hair loss without permanent damage to hair follicles, while scarring alopecias result in permanent destruction of hair follicles due to inflammation and fibrosis (9). Alopecia is a well-known clinical sign of hereditary vitamin D resistant rickets (HVDRR), a rare disease caused by mutations in VDR. Growing evidence indicates that VDR plays a crucial role in normal hair cycling (10, 11). However, the relationship between blood vitamin D levels, tissue vitamin D concentrations, and VDR function remains to be researched since the impact of vitamin D levels on VDR function is complex and may depend on receptor sensitivity, co-regulators, and target gene expression (12). Although it is unknown whether or not deficient vitamin D levels in the blood would lead to deficient vitamin D in the tissue and whether or not this would lead to VDR dysfunction, numerous studies have linked VDD to the pathogenesis of various alopecia disorders, with a focus on AA, male androgenetic alopecia (MAGA), and female pattern hair loss (FPHL). While these studies demonstrate varying degrees of association between VDD and alopecia, there is still debate over the causality and consistency of findings (13–16). We aimed to conduct a systematic review and meta-analysis to determine the prevalence of VDD among various alopecia disorders, namely AA, AGA, TE, and PCA, the odds of VDD and differences in vitamin D levels of patients with various alopecia disorders compared to controls, and whether vitamin D levels are correlated with the severity of alopecia.

2 Materials and methods

2.1 Study design

The protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews; CRD42023387901, https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=387901). The systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines (Supplementary Document 1) (17). Electronic searches were conducted from the database's inception to September 2024 using the PubMed, Embase, Scopus, and Cochrane Library databases. The search strategy was designed to retrieve all studies on vitamin D, VDD, and alopecia using keywords and a controlled vocabulary. There were no restrictions on the language or publication period of the searches. Conference abstracts were excluded. The search included a combination of terms: 'vitamin D,' 'vitamin D deficiency', 'alopecia,' and 'hair loss;' with synonyms, related terms, and subject headings also used. Boolean operators (AND, OR) were used to combine terms. Grey literature and unpublished data were not considered. Supplementary Table S1 provides details about the search strategy.

2.2 Study selection

Two reviewers (TY and KaT) independently evaluated each article at both the full-text and title/abstract levels. Disagreements between the two reviewers regarding the studies' eligibility were resolved via discussion with a third reviewer (PS). We included randomized controlled trials, cohort studies, and case–control studies that provided data for the calculation of the odds ratio (OR) of VDD, the mean difference in vitamin D level between cases and controls, the prevalence of VDD among patients with certain alopecia disorders, or the correlation coefficient (CC) between vitamin D level and alopecia severity. Studies involving patients without a confirmed diagnosis of alopecia or those taking vitamin D supplements were excluded to ensure consistency in the data. Our review questions in the format of PICO (population, intervention, comparator, and outcomes) are provided in Supplementary Table S2. We excluded studies that did not provide a specific type diagnosis of alopecia as well as those that included patients taking vitamin D supplements.

2.3 Data extraction

Data were extracted from the included studies using a standardized form. The following data were collected: bibliographic data (authors, year of publication), study characteristics (type of study, single or multicenter, study duration, country), alopecia group characteristics (number, age, gender, Fitzpatrick skin type (FST), ethnicity, body mass index (BMI), comorbidity, smoking status, alcohol consumption status, diet, sun-exposure, sunscreen usage, disease duration, disease severity score or grading (e.g., mean Severity of Alopecia Tool (SALT) score), alopecia pattern (e.g., % single patch, % multiple patches, % patchy type, % ophiasis type, % alopecia totalis (AT), % alopecia universalis (AU), % body site involvement), family history of alopecia, % nail involvement, % first episode, % recurrent episode, % stable/gradual disease, % active disease, treatment information, whether the diagnosis and severity assessment were done by dermatologist), control group characteristics (number, age, gender, FST, ethnicity, BMI, comorbidity, smoking status, alcohol consumption status, diet, sun

exposure, sunscreen usage, whether controls were matched for any relevant factors), vitamin D results data (frequency data of VDD, vitamin D level, correlation coefficient, and relevant descriptive data), vitamin D measurement (VDD definitions, if any conversion was done, measurement methods), and other (exclusion criteria, if any conversion or data retrieval was done). Vitamin D levels were reported in various units across studies (ng/mL and nmol/L). To maintain consistency, all vitamin D values were converted to ng/mL using standard conversion methods. This ensured comparability of results across different vitamin D assays used in the included studies.

Because $1,25(OH)_2D_3$ has a half-life of less than 4 h and the levels may remain normal in VDD, whereas 25(OH)D has a half-life of approximately 2 weeks, 25(OH)D is a stable indicator of vitamin D status and is routinely measured (18). In this review, the vitamin D level is therefore referred to as the 25(OH)D level.

Corresponding investigators were contacted via email if there was missing data. Two independent reviewers (TY and KaT) extracted data, and discrepancies were resolved with the assistance of a third reviewer (PS).

2.4 Quality assessment

TY and KaT independently assessed the quality of descriptive and case–control studies using the adapted version of the Newcastle-Ottawa Scale (NOS) (19). The NOS is a scoring tool comprised of seven items with nine scores that assess how well the investigators selected their participants (score ranges from 0 to 4), the comparability of their results (score ranges from 0 to 2), and the applicability of the outcomes (score ranges from 0 to 3). The higher the score, the higher the study's quality and the lower the likelihood of bias. Therefore, we classified studies as having high quality if they received a total score of 7 or more, fair quality if they received a score of 4–6, and low quality if they received a score of less than 4. Any discrepancies between reviewers regarding the risk of bias in specific studies were resolved through discussion with a third reviewer (PS). The modified NOS used in our review is shown in Supplementary Table S3.

2.5 Statistical analysis

A meta-analysis was performed to pool the effect sizes, including the OR of a certain alopecia disorder and VDD, the unstandardized mean difference (UMD) of serum vitamin D level between subjects with a certain alopecia disorder and those without, the CC between vitamin D level and the SALT score. Additionally, the "metaprop" command with the Freeman-Tukey double arcsine transformation to stabilize the variances was used in Stata to pool the prevalence of VDD among various alopecia disorders (20). Each alopecia disorder was analyzed separately, and data from adult and pediatric populations was pooled independently. However, as a limited number of studies of scarring alopecia were expected, primary scarring alopecia diseases were planned to be analyzed based on their etiology, such as lymphocytic, neutrophilic, and mixed cell scarring alopecia (9).

Heterogeneity was assessed and considered present if a Cochrane Q test p-value was <0.1 or Higgins $I^2 \geq 25\%$ (21). Subgroup analyses were further performed to explore potential sources of heterogeneity. Effect sizes were pooled using the DerSimonian and Laird method if they were heterogeneous; otherwise, the inverse-variance method was used (21). The sources of heterogeneity were explored by fitting each covariate (e.g., age, female gender, disease duration, BMI, active disease, relapse disease, severe AA, and SALT score) at a time in a meta-regression model. If the $\tau 2$ was decreased by ≥50% or statistically significant β was revealed, a subgroup analysis was performed based on that covariate (22). In addition, certain pre-planned subgroup analyses (country of research origin, age group, and alopecia severity) were also performed. Severe AA is defined as AT, AU, or extensive AA, and an AA cohort is considered severe AA if it has a mean SALT score ≥ 50% or ≥ 20% severe AA. We also conducted sensitivity analyses including only studies with high quality according to the NOS (studies receiving a total NOS score of 7 or more).

To evaluate publication bias, Deeks funnel plots of the primary outcomes were generated. The Egger linear regression test was applied when a funnel plot suggested possible asymmetry (23). If Egger's test for a regression intercept gave a p-value <0.05, a contour-enhanced funnel plot was used to determine the cause of the asymmetry (23). STATA 16.0 (StataCorp LLC, College Station, TX, United States) was used for all statistical analysis.

3 Results

3.1 Study characteristics

After removing duplicates, 2010 references were screened by title/abstract. At the full-text stage, 153 full articles met our predefined selection criteria and were sought. We further excluded 83 references for the following reasons: conference abstracts ($n = 29$), not outcome of interest (i.e., no documented vitamin D deficiency prevalence or vitamin D level of the patients, $n = 30$), not population of interest (i.e., non-specific alopecia diagnosis and specialized hair loss disorders [e.g., drug-induced alopecia, hair loss associated congenital disorders, $n = 15$), review articles ($n = 5$), editorial or commentary ($n = 3$), and case report ($n = 1$)] (Figure 1). Twelve additional studies were identified by manually searching reference lists of included studies and relevant review articles, and 1 study was removed due to insufficient data. The review included 81 studies (79 studies were included in the quantitative analysis; 2 studies of pediatric non-scarring alopecia (24, 25) were excluded from the quantitative analysis), enrolling a total of 15,339 patients with alopecia [8,639 AA patients (13–16, 26–69), 2,943 AGA patients (14, 16, 27, 31, 34, 37, 70–86), 3,048 TE patients (27, 31, 34, 37, 78, 81, 84, 87–96), 489 LPP patients (34, 37, 97, 98), 107 FFA patients (37, 70), and 113 CCCA patients (34, 37, 99, 100)] between 2011 and 2024, were included in the review. Characteristic features of the included studies are provided in Tables 1–4 and Supplementary Table S4.

3.2 Alopecia areata

Patients with AA were found to have a pooled prevalence of VDD of 51.94% (95% confidence interval: 41.54–62.25%,

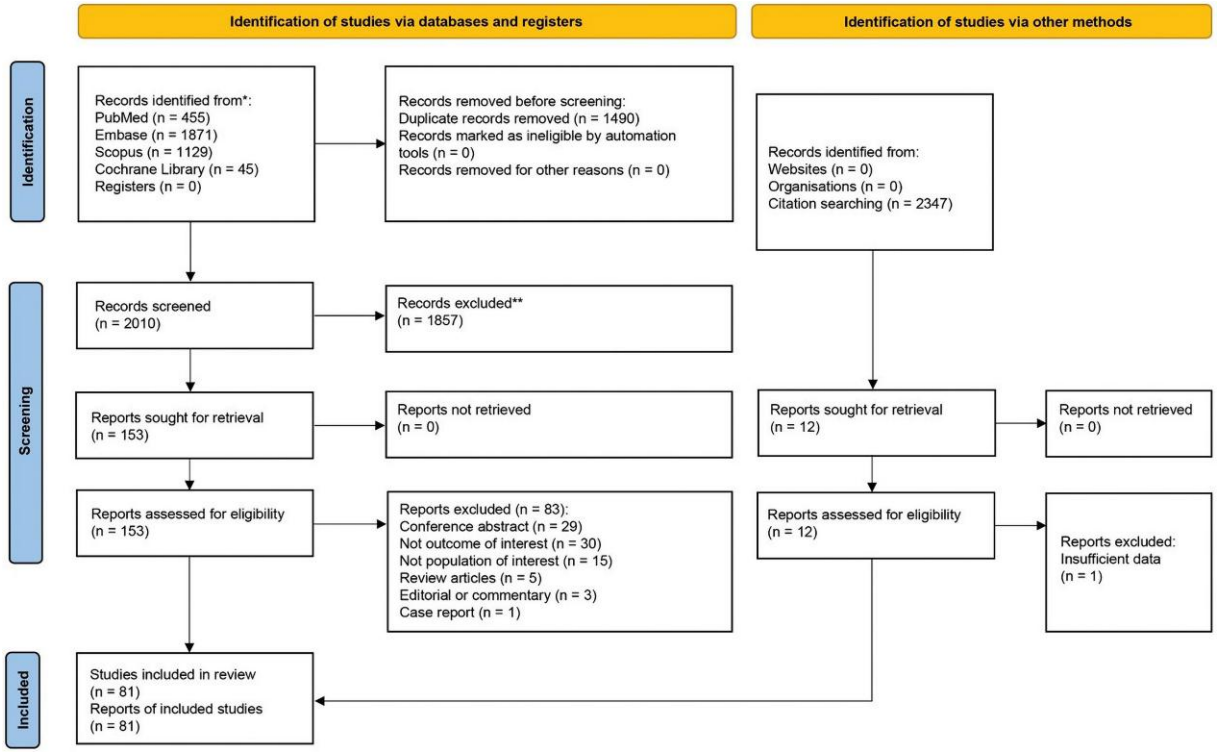

FIGURE 1
Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of search strategy and included studies.

I^2 = 97.48%, p < 0.01), a pooled OR VDD of 2.84 (1.89–4.26, I^2 = 84.29%, p < 0.01), a pooled UMD of −8.20 ng/mL (−10.28 – −6.12 ng/mL, I^2 = 74.25%, p < 0.01), and a pooled CC of vitamin D level and a SALT score of −0.42 (−0.59 – −0.25, I^2 = 85.00%, p < 0.01), indicating a significantly higher likelihood of VDD in AA patients compared to controls.

Meta-regression analysis revealed that disease duration and relapse may account for heterogeneity in pooled OR analyses, while the female gender may account for heterogeneity in pooled UMD analyses. Subsequent subgroup analyses revealed that a disease duration of 12 months or more had a pooled OR of 2 (1.04–3.83, I^2 = 73.87%, p < 0.01), whereas a disease duration of less than 12 months had a pooled OR of 11.53 (5.55–23.96, I^2 = 46.31%, p = 0.13). The pooled OR for cohorts with relapse AA of 50% or more was 8.19 (1.92–35.01, I^2 = 71.52%, p = 0.03), while the pooled OR for cohorts with relapse AA of less than 50% was 3.62 (1.74–7.55, I^2 = 76.96%, p < 0.01), which was statistically significant (p < 0.01). Cohorts with less than 50% female had a pooled UMD of −8.44 ng/mL (−10.49 – −6.39 ng/mL, I^2 = 46.78%, p = 0.01), whereas cohorts with more than 50% female had a pooled UMD of −6.94 ng/mL (−10.78–3.11 ng/mL, I^2 = 81.01%, p < 0.01). Figure 2 demonstrates forest plots for the pooled prevalence of VDD (Figure 2A), pooled odds ratio of VDD (Figure 2B), pooled UMD of vitamin D levels (Figure 2C), and pooled CC of vitamin D level and SALT score (Figure 2D) in adult AA. Supplementary Figure S1 shows subgroup analyses based on disease duration, proportion of relapsed AA, and proportion of females in adult AA.

3.3 Pediatric alopecia areata

A pooled VDD prevalence and a pooled VDD OR of 38.25% (7.32–75.68%, I^2 = 98.35%, p < 0.01) and 3.50 (0.59–20.89, I^2 = 94.02%, p < 0.01) were found, respectively. Figure 3 displays forest plots for the pooled prevalence (Figure 3A) and pooled odds ratio of VDD (Figure 3B) in pediatric AA.

3.4 Androgenetic alopecia

A pooled VDD prevalence of 47.27% (32.49–62.29%, I^2 = 96.06%, p < 0.01) was found for AGA, while a pooled VDD OR of 3.43 (0.95–12.35, I^2 = 94.29%, p < 0.01) and a pooled UMD of vitamin D levels of −6.39 ng/mL (−9.81 – −2.97 ng/mL, I^2 = 88.56%, p < 0.01) were found for AGA compared to controls. For FPHL, a pooled VDD prevalence of 50.38% (31.56–69.14%, I^2 = 93.60%, p < 0.01) was found. Also, a pooled VDD OR of 5.24 (1.50–18.33, I^2 = 81.65%, p < 0.01) and a pooled UMD of vitamin D levels of −15.67 ng/mL (−24.55 – −6.79 ng/mL, I^2 = 91.60%, p < 0.01) were found compared to controls, showing a strong association between VDD and FPHL. For MAGA, a pooled VDD prevalence of 47.38% (20.41–75.17%, I^2 = 94.16%, p < 0.01) was found. For MAGA, a pooled VDD OR of 4.42 (0.53–36.61, I^2 = 88.40%, p < 0.01), and a pooled UMD of vitamin D levels of −2.19 ng/mL (−4.07 – −0.31 ng/mL, I^2 = 7.64%, p < 0.37) were found compared to controls. Figure 4 depicts forest plots for the pooled prevalence of VDD (Figure 4A), pooled odds ratio of VDD (Figure 4B), and pooled UMD of vitamin D levels (Figure 4C) in AGA.

TABLE 1 Characteristics of the included studies involving patients with alopecia areata.

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)	Severity of alopecia
Hasanbeyzade and Tunca (2024) (68)	Turkey	Case–control	Patients with AA (41)	26.80 (7.00)	6 (14.60)	AT 10 (24.4%), AU 9 (22.0%), patchy AA 11 (26.8%), diffuse AA 11 (26.8%)
			Age- and sex-matched healthy controls (41)	26.90 (6.90)	5 (12.20)	–
AbdElneam et al. (2024) (63)	Saudi Arabia	Case–control	Patients with AA (82)	25 (3.90)	40 (48.80)	Localized patchy 38 (46.4%), Multiple patchy 31 (37.8%), Ophiasis 13 (15.8%)
			Age-matched healthy controls (81)	23.8 (2.80)	45 (55.60)	–
Saleem et al. (2023) (69)	Pakistan	Case–control	Patients with AA (45)	22.94 (7.92)	18 (40)	SALT class; S1 = 7 (15.6%), S2 = 10 (22.2%), S3 = 18 (40%), S4 = 3 (6.7%), S5 = 7 (15.6%), mean SALT score 56.3%[§]
			Age- and sex-matched healthy controls (45)	23.84 (8.46)	18 (40)	–
Hamidpour et al. (2023) (67)	Iran	Descriptive	Patients with AA (402)	27.20 (13.40)	192 (47.80)	Median SALT score 68 (IQR 40–100)
Gupta et al. (2023) (66)	India	Case–control	Patients with AA (25)	27.64 (9.83)	8 (32)	SALT class; S1 = 20 (80%), S2 = 2 (8%), S3-5 = 3 (12%)
			Age- and sex-matched healthy controls (25)	28.56 (7.95)	8 (32)	–
Fahim et al. (2023) (65)	Pakistan	Descriptive	Patients with AA (100)	30.50 (8.40)	58 (58)	Mean SALT score 20.7 ± 5.4
Alsenaid et al. (2023) (64)	Saudi Arabia	Case–control	Patients with AA (59)	27.10 (9.10)	6 (10.20)	Moderate 6 (10.2%), severe 9 (15.3%)
			Age-matched healthy controls (60)	27.4 (10.30)	9 (15)	–
Das (2022) (26)	India	Case–control	Patients with AA (50)	25.07 (7.40)	18 (36)	SALT class; S1 = 35 (70%), S2 = 10 (20%), S3 = 5 (10%), mean SALT score 22.3%[§]
			Age- and sex-matched healthy controls (50)	24.48 (6.30)	20 (40)	–
deQueiroz et al. (2022) (27)	Brazil	Case–control	Patients with AA (7)	44.2 (14.90)	7 (100)	NR
			Unmatched controls with other skin conditions (33)	38.8 (16.00)	37 (100)	–
Gao et al. (2022) (28)	China	Case–control	Patients with AA (672)	31.28 (14.42)	276 (41.08)	NR
			Age- and sex-matched healthy controls (580)	30.89 (13.00)	238 (41.03)	–
Goksin (2022) (29)	Turkey	Descriptive	Patients with AA (218)	27.8 (12.30)	84 (38.5)	AU 7 (3.2%), AT 1 (0.5%)
Lim et al. (2022) (30)	USA	Descriptive	Patients with pediatric AA (96)	9 (4.40)	61 (64)	NR
Oner and Akdeniz (2022) (31)	Turkey	Descriptive	Patients with AA (99)	26.1 (12.3)	25 (25.3)	NR
Tran et al. (2022) (16)	USA	Case–control*	Patients with AA (417)	45.70 (NR)	561 (61.60)	NR
			Age-, sex-, and race-matched patients (3127)	49.40	3,685 (74.50)	–
Abedini et al. (2021) (32)	Iran	Case–control	Patients with AA (50)	32.48 (12.61)	23 (46)	Ophiasis 6 (12%), AT 9 (18%), AU 18 (36%)
			Age, sex, and BMI-matched healthy controls (50)	32.26 (12.32)	23 (46)	–
Alamoudi et al. (2021) (33)	Saudi Arabia	Descriptive	Patients with AA (177)	28.37 (12.68)	92 (52)	AU 16 (9%), AT 23 (7%)

(Continued)

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TABLE 1 (Continued)

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)	Severity of alopecia
Conic et al. (2021) (34)	USA	Descriptive	Patients with AA (77)	37.2‡ (NR)	54 (70.10)	NR
Lizarondo et al. (2021) (35)	Philippines	Case–control	Patients with AA (29)	31.48 (10.82)	19 (65.5)	SALT class; S1 = 20 (68.97%), S2 = 5 (17.24%), S3 = 2 (6.90%), S4 = 2 (6.90%), mean SALT score 25.24%§
			Age-, sex-, and sun exposure per day-matched healthy controls (29)	31.86 (10.51)	19 (65.5)	–
Conic et al. (2020) (36)	USA	Case–control*	Patients with pediatric AA (3510)	NR	1940 (55.3)	NR
			Unmatched pediatric controls without AA (8310710)	NR	4,018,940 (48.4)	–
Zhao et al. (2020) (14)	China	Case–control	Patients with AA (443)	41.26 (14.10)	279 (62.98)	NR
			Age-, sex-, and season-matched healthy controls (2070)	41.76 (11.25)	1,006 (48.60)	–
Conic et al. (2019) (37)	USA	Descriptive	Patients with AA (18)	71.83 (6.34)	15 (83.3)	NR
El-Ghareeb (2019) (38)	Egypt	Case–control	Patients with AA (20)	NR	NR	NR
			Age-matched healthy controls (20)	NR	NR	–
Marahatta et al. (2019) (39)	Nepal	Case–control	Patients with AA (30)	28.37 (10.07)	14 (48.3)	SALT score = 3.56 ± 3.50%
			Age- and sex-matched healthy controls (30)	30.50 (9.03)	15 (51.7)	–
Namdar and Arikan (2019) (40)	Turkey	Case–control	Patients with AA (60)	31.4 (10.03)	30 (50)	SALT class; S1 = 43 (71.7%), S2 = 17 (28.3%), mean SALT score 19.44%§
			Unmatched controls without chronic or dermatological diseases (61)	36.61 (10.08)	27 (44.3)	–
Rehman et al. (2019) (41)	India	Case–control	Patients with AA (135)	26 (12.89)	44 (32.59)	SALT class; S1 = 52 (38.52%), S2 = 35 (25.93%), S3 = 17 (12.59%), S4 = 11 (8.15%), S5 = 7 (5.19%), mean SALT score 38.09%§
			Age- and sex-matched healthy controls (135)	26 (13.20)	44 (32.59)	–
Siddappa et al. (2019) – adult AA (42)	India	Case–control	Patients with AA (100)	24.52 (10.06)	28 (28)	SALT class; S1 = 75 (99%), S3 = 1 (1%), mean SALT score 13.14%§
			Age- and sex-matched healthy controls (100)	28.96 (11.49)	42 (42)	–
Siddappa et al. (2019) – pediatric AA (43)	India	Case–control	Patients with pediatric AA (30)	11.13 (4.17)	12 (40)	NR
			Age- and sex-matched healthy controls (30)	11.46 (4.41)	12 (40)	–
Daroach et al. (2018) (44)	India	Case–control	Patients with AA (30)	28.97 (9.96)	19 (63.33)	SALT class; S1-2 = 24 (80%), S3-4 = 3 (10%), S5 = 3 (10%), SALT score = 35.8 ± 27.5%
			Age- and sex-matched healthy controls (30)	31.17 (9.43)	14 (46.67)	–
Gade et al. (2018) (45)	India	Case–control	Patients with AA (45)	32.73 (10.43)	31 (68.89)	Median SALT score (%) 4.23 (3.12–6.33)
			Age- and sex-matched healthy controls (45)	33.98 (8.48)	31 (68.89)	–

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TABLE 1 (Continued)

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)	Severity of alopecia
Karaguzel et al. (2018) (46)	Turkey	Case–control	Patients with pediatric AA (30)	10.5 (2.9)	20 (66)	NR
			Age- and sex-matched healthy pediatric control (30)	10.5 (2.8)	20 (66)	–
Saniee et al. (2018) (15)	Iran	Case–control	Patients with AA (77)	27.38 (11.94)	37 (48.1)	Mean involved area = 43.51 ± 20.25
			Age- and sex-matched normal controls who visited dermatology clinics (112)	29.54 (13.65)	54 (48.2)	–
Unal and Gonulalan (2018) (47)	Turkey	Case–control	Patients with pediatric AA (20)	12.67 (4.16)	6 (30)	SALT class; S1 = 6 (30%), S2 = 9 (45%), S3 = 5 (25%), S4 = 0, S5 = 0, mean SALT score 35.78%[§]
			Unmatched healthy controls (34)	16.54 (0.91)	19 (55.88)	–
Bhat et al. (2017) (48)	India	Case–control	Patients with AA (50)	22.4 (8.6)	NR	SALT class; S1 = 38 (76%), S2 = 12 (24%), mean SALT score 18.38%[§]
			Age- and sex-matched healthy controls randomly recruited from clinic with no history of AA (35)	29.2 (7.6)	NR	–
Conic et al. (2017) (49)	USA	Case–control*	Patients with AA (584)	35.54 (19.28)	400 (68.50)	AT 12 (2.05%), AU 19 (3.25%)
			Age-matched controls with seborrheic dermatitis without hair loss (172)	35.80 (15.56)	126 (73.25)	–
Erpolat et al. (2017) (50)	Turkey	Case–control	Patients with AA (41)	32.8 (7.5)	15 (36.59)	NR
			Unmatched healthy controls (32)	32.7 (7.5)	14 (43.75)	–
Ghafoor and Anwar (2017) (51)	Pakistan	Case–control	Patients with AA (30)	23.77 (8.86)	18 (60)	SALT class; S1 = 4 (13.33%), S2 = 7 (23.33%), S3 = 12 (40%), S4 = 1 (3.33%), S5 = 6 (20%), mean SALT score 57.8%[§]
			Age- and sex-matched healthy volunteers and patients coming to dermatology department for other disorders (30)	24.03 (8.62)	18 (60)	–
Narang et al. (2017) (52)	India	Descriptive	Patients with AA (22)	30.4 (10.8)	10 (45.5)	SALT score ranged 8.4–40
Attawa et al. (2016) (53)	Egypt	Case–control	Patients with AA (23)	26.44 (10.87)	8 (34.8)	SALT class; S1 = 14 (61%), S2 = 3 (13%), S3 + S4 + S5 = 6 (26%), mean SALT score 34.04%[§]
			Unmatched healthy controls (23)	29.39 (8.10)	9 (39.1)	–
Bakry et al. (2016) (54)	Egypt	Case–control	Patients with AA (60)	20.7 (10.85)	24 (40)	Ophiasis 12 (20%), AT/AU 16 (26.7%)
			Age-, sex-, FST-, and BMI-matched healthy controls (60)	23.71 (7.45)	32 (53.3)	–
Darwish et al. (2016) (55)	Egypt	Case–control	Patients with AA (30)	28.67 (10)	17 (56.7)	SALT score: S1 = 10 (33.3%), S2 = 7 (23.3%), S3 = 4 (13.3%), S4 = 3 (10%), S5 = 1 (3.3%), S4 B = 3 (10%), S5 B = 2 (6.7%), mean SALT score 39.64%[§]
			Age- and sex-matched healthy controls (20)	24.8 (6)	10 (50)	–

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TABLE 1 (Continued)

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)	Severity of alopecia
Fattah and Darwish (2015) (56)	Egypt	Case–control	Patients with AA (30)	26.8 (6.9)	12 (40)	SALT class; S3 = 15 (50%), S4 = 3 (10%), S5 = 12 (40%), B0 = 27 (90%), B2 = 3 (10%), mean SALT score 79.45%[§]
			Age-, sex-, FST-, approximate daily amount of vitamin D intake-, occupation (indoor or outdoor)-, and time of blood sampling-matched healthy controls (30)	25.1 (6.9)	12 (40)	–
Ogrum et al. (2015) (57)	Turkey	Case–control	Patients with AA (40)	31.23 (7.34)	21 (52.5)	SALT class; S1 = 35 (87.5%), S2 = 3 (7.5%), S3 = 2 (5%), mean SALT score 16.79%[§]
			Age-, sex-, and FST-matched healthy controls (40)	30.58 (7.19)	21 (52.5)	–
Cerman et al. (2014) (13)	Turkey	Case–control	Patients with AA (86)	32.21 (9.60)	30 (42)	SALT class; S1 = 41 (83%), S2 = 15 (17%), SALT 14.41 ± 9.92%
			Age- and sex-matched healthy controls (58)	32.55 (9.78)	24 (41.38)	–
Mahamid et al. (2014) (58)	Israel	Case–control	Patients with AA (23)	24.2 (12.3)	9 (39.13)	Extensive 5 (21.74%)
			Age- and sex-matched controls without AA (20)	27 (11.26)	7 (35)	–
D'Ovidio et al. (2013) (59)	Italy	Case–control	Patients with AA (70)	27.79 (9.12)	33 (47.1)	Ophiasis 69 (44%), AT/AU 38 (24.5%)
			Unmatched healthy controls (70)	30.49 (11.06)	26 (37.1)	–
El-Mongy et al. (2013) (60)	Egypt	Case–control	Patients with AA (156)	37.8 (NR)	111 (71.15)	SALT class; S1 = 30 (42.9%), S2 = 12 (17.1%), S3 + S4 + S5 = 28 (40.0%), mean SALT score 44.83%[§]
			Unmatched healthy controls (148)	34.5 (NR)	130 (87.84)	–
Nassiri et al. (2013) (61)[‖]	Iran	Case–control	Patients with AA (28)	27.75 (7.97)	9 (32.14)	SALT (%); 0–24 = 6 (21.4%), 25–49 = 4 (14.3%), 50–74 = 1 (3.6%), and 100 = 17 (60.7%), mean SALT score 70.79%[§]
			Unmatched healthy controls (44)	33.16 (12.52)	28 (63.63)	–
Yilmaz et al. (2012) (62)	Turkey	Case–control	Patients with AA (42)	31.1 (8.2)	28 (66.67)	SALT class; S1 = 30 (71.43%), S2 = 6 (14.29%), S3 = 3 (7.14%), S4 = 2 (4.76%), S5 = 1 (2.38%), mean SALT score 25.13%[§]
			Unmatched healthy controls (42)	29.3 (7.4)	29 (69.05)	–

AA, alopecia areata; BMI, body mass index; FST, Fitzpatrick skin type; NR, not reported; SALT, Severity of Alopecia Tool; USA, United States. * Multicenter studies. [‡] Median age. ‖ Vitamin D deficiency defined as < 10 ng/mL. § calculated mean SALT score.

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TABLE 2 Characteristics of the included studies involving patients with androgenetic alopecia.

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)	Severity of alopecia
Wang et al. (2024) (86)	China	Case–control	Patients with MAGA (40)	27.3 (5.30)	0	NR
			Age- and gender-matched healthy controls (45)	28.3 (4.20)	0	–
Losoya-Jaquez et al. (2024) (25)	Mexico	Descriptive	Patients with pediatric AGA (13[†])	16.08 (1.30)	42 (21)	NR
Wu et al. (2023) (85)	China	Case–control	Patients with MAGA (80)	36.28 (10.49)	0	Mild 36 (45%), moderate alopecia 37 (46.3%), severe alopecia 7 (0.09%)
			Age-, gender- and BMI-matched healthy controls (60)	36.28 (10.98)	0	–
Vandana et al. (2023) (84)	India	Descriptive	Patients with FPHL (24)	28.9 (NR)	24 (100)	NR
Okhovat et al. (2023) (83)	USA	Descriptive	Patients with FPHL (54[†])	50.04 (16.40)	54 (100)	NR
Hailat et al. (2023) (82)	Pakistan	Case–control	Patients with FPHL (72)	28.6 (2.40)	72 (100)	NR
			Sex-matched healthy controls (72)	NR	72 (100)	–
Arasu et al. (2022) (70)	Australia	Descriptive	Patients with FPHL (100)	51 (NR)	100 (100)	NR
deQueiroz et al. (2022) (27)	Brazil	Case–control	Patients with FPHL (37)	54.8 (15.00)	37 (100)	NR
			Unmatched controls with other skin conditions (33)	38.8 (16.00)	37 (100)	–
Krysiak et al. (2022) (71)	Poland	Case–control	Patients with MAGA (72)	37 (6.00)	0	NR
			Age-, blood pressure-, BMI-, insulin sensitivity-, and plasma lipids-matched controls without hair loss (75)	38 (6.00)	0	–
Oner and Akdeniz (2022) (31)	Turkey	Descriptive	Patients with AGA (101)	25.6 (7.3)	25 (24.8)	NR
Tran et al. (2022) (16)	USA	Case–control*	Patients with AGA (404)	NR	NR	NR
			Age-, sex-, and race-matched patients (3127)	49.40	3,685 (74.50)	–
Conic et al. (2021) (34)	USA	Descriptive	Patients with AGA (73)	53.2[‡] (NR)	65 (89)	NR
Danane et al. (2021) (72)	India	Descriptive	Patients with MAGA (50)	24 (NR)	0	NR
El-Tahlawy et al. (2021) (73)	Egypt	Case–control	Patients with MAGA (30)	NR	0	NR
			Age- and sex-matched healthy controls (30)	NR	0	–
Jasim et al. (2021) (74)	Iraq	Case–control	Patients with FPHL (50)	NR	50 (100)	NR
			Unmatched healthy controls (50)	NR	50 (100)	–
Kerkemeyer et al. (2021) (75)	Australia	Descriptive	Patients with MAGA (31[†])	28.7 (NR)	0	Sinclair grade; 2.0 = 15 (17.6%), 2.5 = 6 (7.1%), 3.0 = 40 (47.6%), 3.5 = 0 (0.0%), 4.0 = 18 (21.4%), 4.5 = 2 (2.4%), and 5.0 = 3 (3.6%)

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TABLE 2 (Continued)

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)	Severity of alopecia
Sanke et al. (2020) (76)	India	Case–control	Patients with MAGA (50)	21.17 (3.66)	0	Hamilton-Norwood grade; III = 14 (25%), IV = 19 (33%), V = 20 (35%), and grade VI = 4 (7%)
			Age-, sex-, socioeconomic status, and outdoor exposure-matched healthy controls who attended dermatology department (50)	NR	0	–
Zhao et al. (2020) (14)	China	Case–control	Patients with FPHL (657)	32.59 (10.51)	657 (100)	NR
			Patients with MAGA (777)	29.89 (7.02)	0	NR
			Age-, sex-, and season-matched healthy controls (2070)	41.76 (11.25)	1,006 (48.60)	–
Conic et al. (2019) (37)	USA	Descriptive	Patients with FPHL (27)	70.26 (4.99)	27 (100)	NR
Kondrakhina et al. (2019) (77)	Russia	Case–control	Patients with MAGA (50)	26.2 (5.3)	0	NR
			Age- and origin-matched healthy controls (25)	NR	NR	–
Sarac and Koca (2018) (78)	Turkey	Case–control	Patients with AGA (58)	30.3 (8.8)	28 (48.28)	NR
			Unmatched healthy controls (58)	28.5 (10.1)	47 (81.03)	–
Banihashemi et al. (2016) (79)	Iran	Case–control	Patients with FPHL (45)	29.11 (7.31)	45 (100)	NR
			Age-, sex-, hours spent under sunlight per day-, and BMI-matched healthy controls (45)	28.82 (7.11)	45 (100)	–
Moneib et al. (2014) (80)	Egypt	Case–control	Patients with FPHL (60)	28.67 (10)	60 (100)	NR
			Age-, sex-, FPT-, socioeconomic status-, outdoor exposure- matched heatlhy controls (60)	24.8 (6)	60 (100)	–
Rasheed et al. (2013) (81)	Egypt	Case–control	Patients with FPHL (38)	NR	38 (100)	
			Age-, sex-, and FST- matched healthy female controls (40)	30.8 (8.56)	40 (100)	–

AGA, androgenetic alopecia; BMI, body mass index; FPHL, female pattern hair loss; FST, Fitzpatrick skin type; MAGA, male androgenetic alopecia; NR, not reported; USA, United States. * Multicenter studies. † Number of patients with vitamin D results. ‡ Median age.

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TABLE 3 Characteristics of the included studies involving patients with telogen effluvium.

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)
Vandana et al. (2023) (84)	India	Descriptive	Patients with TE (76)	24.40 (NR)	76 (100)
Arslan et al. (2023) (96)	Turkey	Descriptive	Patients with TE (58†)	27.54 (9.42)	840 (86.3)
Chen et al. (2022) (24)	USA	Descriptive	Patients with pediatric TE (68†)	12.3 (NR)	67 (88)
deQueiroz et al. (2022) (27)	Brazil	Case–control	Patients with TE (17)	42.8 (14.55)	17 (100)
			Unmatched controls with other skin conditions (33)	38.8 (16.00)	37 (100)
Oner and Akdeniz (2022) (31)	Turkey	Descriptive	Patients with TE (160)	27.7 (8.8)	156 (97.5)
Yorulmaz et al. (2022) (87)	Turkey	Descriptive	Patients with TE (1688†)	26‡ (NR)	2,794 (92.30)
Alizadeh et al. (2021) (88)	Iran	Case–control	Patients with TE (83)	35‡ (NR)	83 (100)
			Age- and sex-matched healthy controls (83)	35‡ (NR)	83 (100)
Conic et al. (2021) (34)	USA	Descriptive	Patients with TE (121)	46.9‡ (NR)	120 (99.2)
Naser et al. (2021) (89)	Baghdad	Case–control	Patients with TE (60)	32.6 (6.47)	60 (100)
			Age- and sex-matched healthy controls (60)	41.3 (4.59)	60 (100)
Mohammad et al. (2020) (90)	Iran	Case–control	Patients with TE (50)	NR	50 (100)
			Age- and sex-matched healthy controls who referred to dermatology clinic for cosmetic procedures other than hair loss (50)	NR	50 (100)
Sokmen (2020) (91)	Turkey	Descriptive	Patients with TE (151)	29‡ (NR)	151 (100)
Conic et al. (2019) (37)	USA	Descriptive	Patients with TE (70)	71.07 (5.35)	68 (97.1)
Cifcia (2018) (92)	Turkey	Case–control	Patients with TE (155)	30.7 (9.80)	149 (96.13)
			Age- and sex-matched healthy controls who visited other clinics for health checkup (168)	30.76 (8.80)	155 (92.26)
Sarac and Koca (2018) (78)	Turkey	Case–control	Patients with TE (71)	26.6 (8.4)	65 (91.55)
			Unmatched healthy controls (58)	28.5 (10.1)	47 (81.03)
Gurel et al. (2017) (93)	Turkey	Case–control	Patients with TE (80)	26.41 (6.93)	80 (100)
			Age- and sex-matched controls without hair loss (80)	25.79 (7.41)	80 (100)
Cheung et al. (2016) (94)	USA	Descriptive	Patients with TE (115†)	NR	110 (26.63)
Rasheed et al. (2013) (81)	Egypt	Case–control	Patients with TE (42)	NR	42 (100)
			Age-, sex-, and FST- matched healthy female controls (40)	30.8 (8.56)	40 (100)
Karadag et al. (2011) (95)	Turkey	Case–control	Patients with TE (63)	29.1 (11.9)	63 (100)
			Sex-matched controls without AA (50)	28.4 (9.4)	50 (100)

FST, Fitzpatrick skin type; TE, telogen effluvium; USA, United States. † Number of patients with vitamin D results. ‡ Median age.

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TABLE 4 Characteristics of the included studies involving patients with scarring alopecia.

Author, year	Country	Study design	Group (case/control)	Mean age (SD)	Female (%)	Severity of alopecia
Leung et al. (2023) (100)	USA	Case–control	Patients with CCCA (53)	51.3 (9.60)	53 (100)	NR
			Age- and sex-matched healthy controls (212)	50.3 (9.50)	212 (100)	–
Gharaei Nejad et al. (2023) (98)	Iran	Descriptive	Patients with LPP (60)	43.60 (10.17)	44 (73.3)	NR
Arasu et al. (2022) (70)	Australia	Descriptive	Patients with FFA (100)	63 (NR)	100 (100)	NR
Collins et al. (2022) (99)	USA	Descriptive	Patients with CCCA (27)	NR	NR	NR
Conic et al. (2021) (34)	USA	Descriptive	Patients with LPP (58)	56.6[†] (NR)	55 (94.8)	NR
			Patients with CCCA (29)	55.2[†] (NR)	29 (100)	NR
Conic et al. (2019) (37)	USA	Descriptive	Patients with LPP (37)	69.24 (3.11)	37 (100)	NR
			Patients with FFA (7)	68.86 (3.24)	7 (100)	NR
			Patients with CCCA (4)	67.25 (1.26)	4 (100)	NR
Brankov et al. (2018) (97)	USA	Case–control	Patients with LPP (334)	54.77 (12.83)	311 (93.1)	NR
			Age- and race-matched controls with seborrheic dermatitis without hair loss (78)	52.19 (15.37)	62 (79.5)	–

CCCA, central centrifugal cicatricial alopecia; FFA, frontal fibrosing alopecia; LPP, lichen planopilaris; USA, United States. [†]Median age.

3.5 Telogen effluvium

A pooled VDD prevalence of 53.51% (37.33–69.33%, $I^2 = 97.99\%$, $p < 0.01$) was found for TE patients. A pooled VDD OR of 1.14 (0.65–1.98, $I^2 = 48.09\%$, $p = 0.10$) and a pooled UMD of vitamin D levels of −5.71 ng/mL (−10.10 – −1.32 ng/mL, $I^2 = 92.46\%$, $p < 0.01$) were found compared to controls. Figure 5 shows forest plots for the pooled prevalence of VDD (Figure 5A), pooled odds ratio of VDD (Figure 5B), and pooled UMD of vitamin D levels (Figure 5C) in TE.

3.6 Primary scarring alopecia

A pooled VDD prevalence of 38.85% (24.29–54.40%, $I^2 = 91.73\%$, $p < 0.01$) was found for PCA. Subgroup analysis of specific PCA disorders was performed, and pooled VDD prevalences of 18.00% (10.57–26.61%), 56.70% (10.23–97.15%), and 37.04% (25.47–49.39%) were found for FFA, CCCA, and LPP, respectively. Figure 6 illustrates forest plot for pooled prevalence of VDD in PCA.

3.7 Country of research origin subgroup analysis

Pooled VDD prevalences of AA, AGA, and TE in eastern countries of 56.70% (43.04–69.87%, $I^2 = 97.88\%$, $p < 0.01$), 64.31% (48.41–78.81%, $I^2 = 92.10\%$, $p < 0.01$), and 64.42% (49.17–78.33%, $I^2 = 97.10\%$, $p < 0.01$), were found, respectively. Whereas pooled VDD prevalences of 31.36% (23.51–39.74%, $I^2 = 82.73\%$, $p < 0.01$), 25.54% (16.80–35.34%, $I^2 = 82.88\%$, $p < 0.01$), and 27.56% (13.59–44.07%, $I^2 = 88.11\%$, $p < 0.01$) were found for AA, AGA, and TE in western countries.

Pooled VDD ORs of AA, AGA, and TE in eastern countries of 3.18 (2.04–4.97, $I^2 = 75.74\%$, $p < 0.01$), 5.65 (1.75–18.18, $I^2 = 79.96\%$, $p < 0.01$), and 1.04 (0.56–1.94, $I^2 = 53.62\%$, $p = 0.09$) were found, respectively. While pooled VDD ORs of 1.78 (0.77–4.16, $I^2 = 91.62\%$, $p < 0.01$), 1.00 (0.18–5.59, $I^2 = 91.52\%$, $p < 0.01$), and 1.97 (0.60–6.46) were found for AA, AGA, and TE in western countries.

Pooled UMDs of vitamin D levels of AA, AGA, and TE in eastern countries of −8.24 ng/mL (−10.02 – −6.46 ng/mL, $I^2 = 58.11\%$, $p < 0.01$), −8.13 ng/mL (−11.97 – −4.29 ng/mL, $I^2 = 87.64\%$, $p < 0.01$), and − 6.07 ng/mL (−11.02 – −1.13 ng/mL, $I^2 = 93.47\%$, $p < 0.01$) were found, respectively. However, pooled UMDs of vitamin D levels of −6.67 ng/mL (−23.12–9.77 ng/mL, $I^2 = 85.29\%$, $p < 0.01$), and − 0.52 ng/mL (−8.58–7.54 ng/mL, $I^2 = 84.55\%$, $p < 0.01$) were found for AA and AGA in western countries. Table 5 summarizes subgroup analyses based on the country of research origin.

3.8 Age group subgroup analysis

AA and AGA cohorts with mean ages 18–25 years were found to have pooled VDD prevalences of 47.01% (1.32–96.78%) and 70.69% (51.32–86.96%), respectively, while AA and AGA cohorts with mean ages 25–60 years were found to have pooled VDD prevalences of

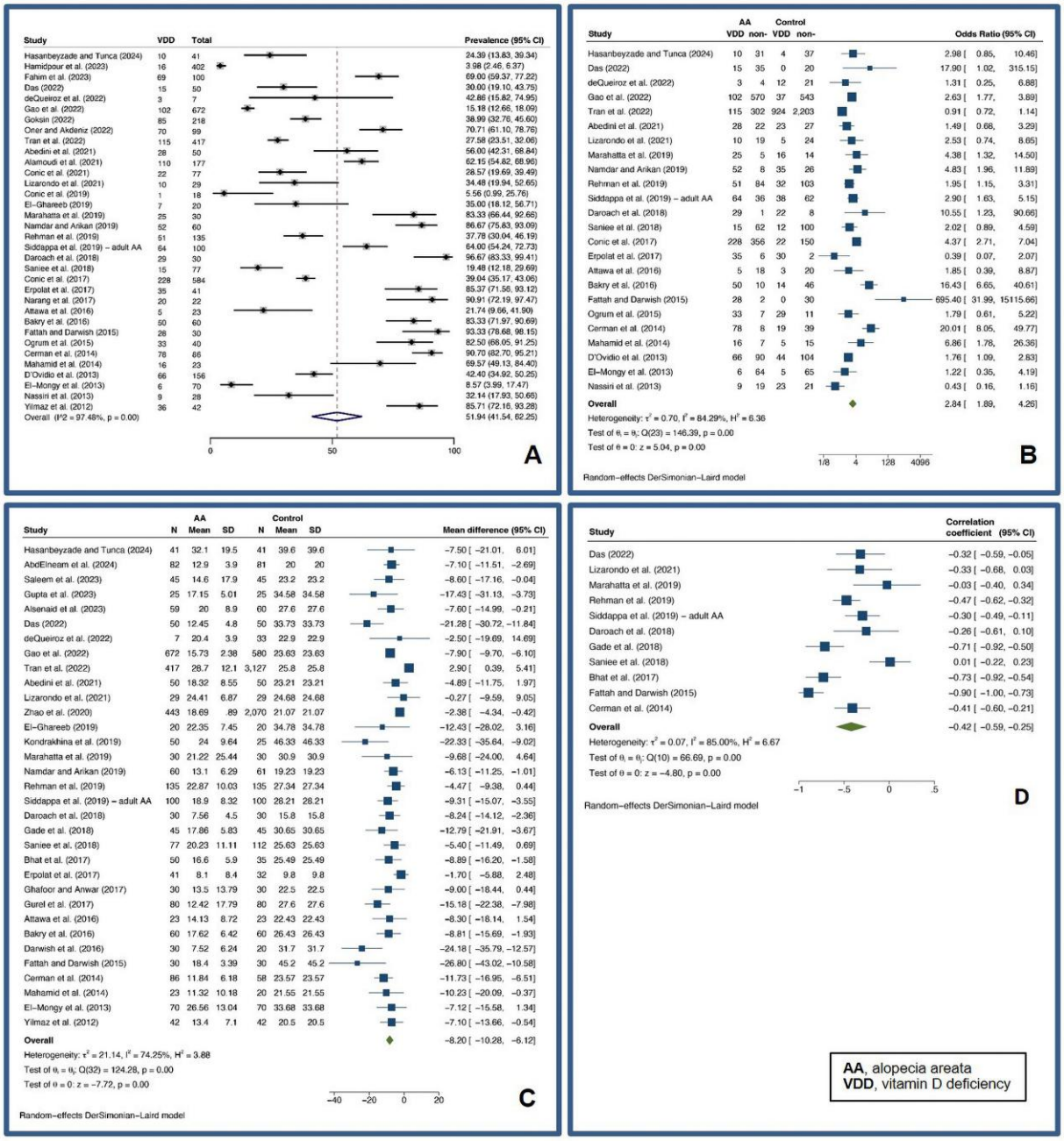

FIGURE 2
Forest plots for the pooled prevalence of vitamin D deficiency **(A)**, pooled odds ratio of vitamin D deficiency **(B)**, pooled unstandardized mean difference of vitamin D levels **(C)**, and pooled correlation coefficient of vitamin D level and Severity of Alopecia Tool score **(D)** in adult alopecia areata.

54.67% (44.56–64.60%, $I^2 = 96.79$, $p < 0.01$) and 42.07% (25.49–59.60%, $I^2 = 96.42$, $p < 0.01$), respectively.

AA cohorts with mean age 18–25 years were found to have a pooled VDD OR of 6.65 (1.22–36.35, $I^2 = 90.06$, $p < 0.01$), while AA cohorts with mean age 25–60 years were found to have a pooled VDD OR of 2.57 (1.70–3.88, $I^2 = 82.36\%$, $p < 0.01$). AA cohorts with mean age 18–25 years were found to have a pooled UMD of vitamin D levels of −8.98 ng/mL (−12.23 – −5.73 ng/mL, $I^2 = 0.00$, $p = 1.00$); however, AA cohorts with mean age 25–60 years were found to have a pooled UMD of vitamin D levels of −8.07 ng/mL (−10.44 – −5.70 ng/mL, $I^2 = 78.12$, $p < 0.01$). Table 5 presents subgroup analyses based on age group.

3.9 Alopecia severity subgroup analysis

Severe AA cohorts were found to have a VDD prevalence of 44.36% (19.70–70.54%, $I^2 = 98.01\%$, $p < 0.01$), VDD OR of 3.29 (1.30–8.34, $I^2 = 85.53\%$, $p < 0.01$), a UMD of vitamin D levels of −10.65 ng/mL (−15.23 – −6.39 ng/mL, $I^2 = 44.83\%$, $p = 0.08$), while non-severe AA cohorts were found to have a VDD prevalence of 63.71% (47.43–78.58%, $I^2 = 95.25\%$, $p < 0.01$), VDD OR of 3.58 (2.20–5.82, $I^2 = 60.92\%$, $p < 0.01$), and a UMD of vitamin D levels of −8.17 ng/mL (−9.97 – −6.37 ng/mL, $I^2 = 15.12\%$, $p = 0.28$). Due to insufficient information, subgroup analyses based on other alopecia disorders were not conducted. Table 5 shows subgroup analyses based on alopecia severity.

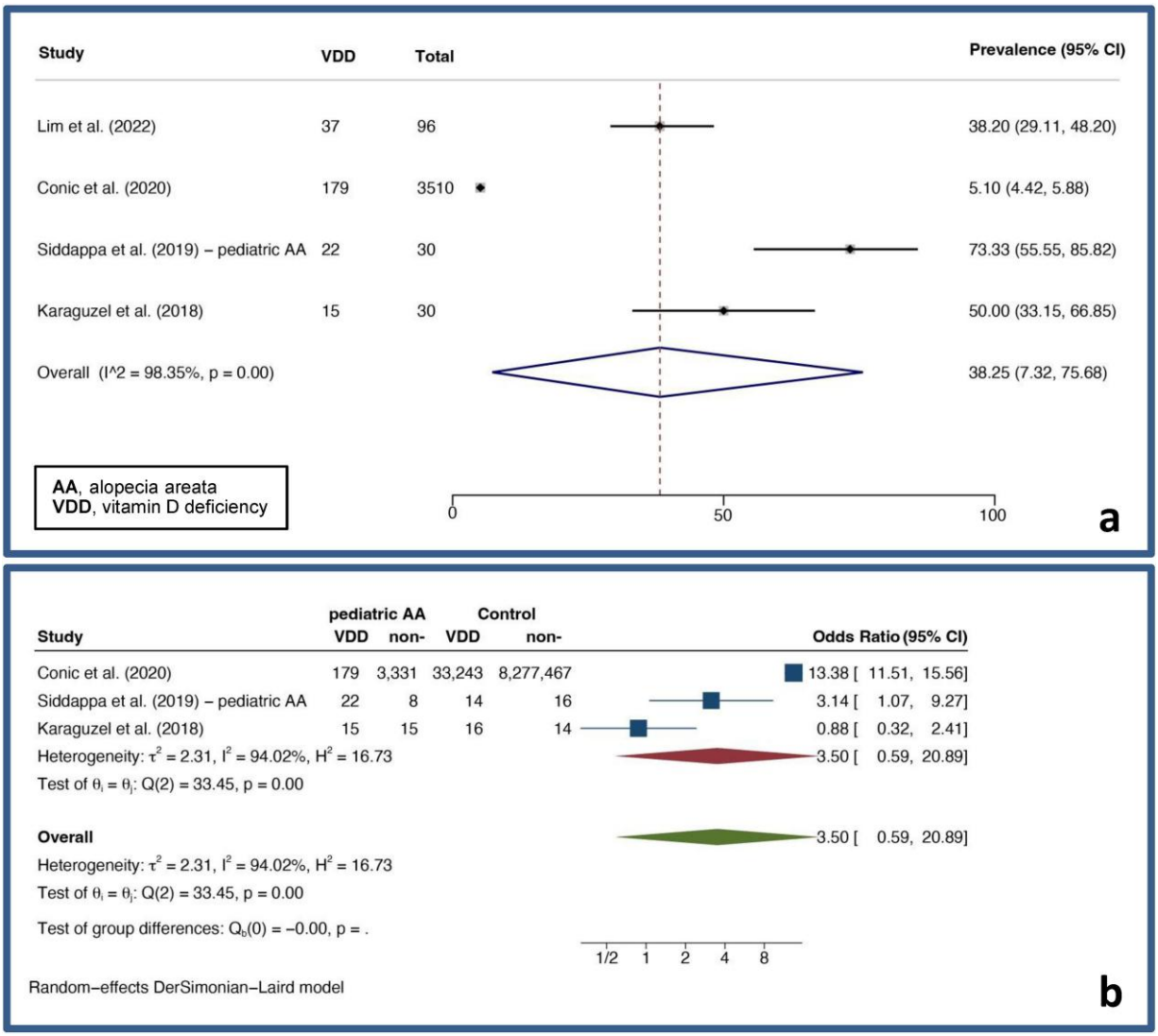

FIGURE 3
Forest plots for the pooled prevalence of vitamin D deficiency **(A)** and pooled odds ratio of vitamin D deficiency **(B)** in pediatric alopecia areata.

3.10 Quality assessment

Supplementary Table S3 provides a summary of the quality assessment scores for comparative and descriptive studies included in the review. The average quality assessment score was 7.22 (range: 5–9), with 59 high-quality and 22 fair-quality studies. Sensitivity analyses based on study quality were performed to assess the robustness of the findings. The results were consistent with the primary analyses, suggesting that potential biases did not significantly influence the pooled estimates (Supplementary Document 2).

3.11 Publication bias

Some funnel plots were slightly asymmetric when assessing publication bias for each primary analysis (Supplementary Figure S2). As a result, Egger's tests were conducted, and it was discovered that some analyses exhibited possible asymmetry; consequently, we performed additional contour-enhanced funnel plots. We discovered that the asymmetry in the VDD OR analyses for AA and AGA, UMD of vitamin D levels analysis in TE and AA, and CC of vitamin D level and a SALT score were likely due to heterogeneity. In the UMD of vitamin D levels analysis in AGA, however, publication bias is highly likely. Funnel plots and contour-enhanced funnel plots are shown in Supplementary Figure S2.

4 Discussion

Our analysis revealed that VDD was prevalent among patients with various alopecia disorders, including AA, FPHL, MAGA, TE, and PCA. Statistically significant associations were observed in AA and FPHL patients, who demonstrated a higher likelihood of VDD and lower vitamin D levels compared to controls, although MAGA

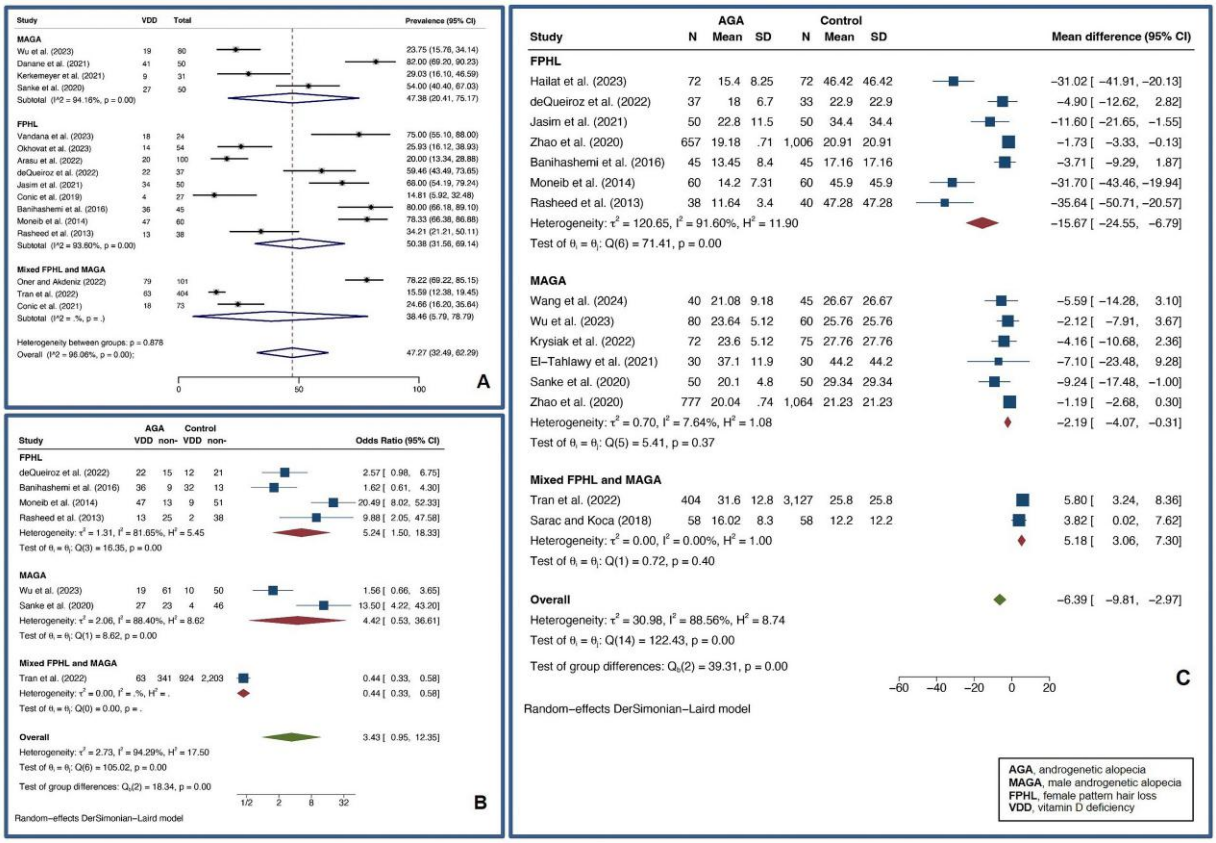

FIGURE 4
Forest plots for the pooled prevalence of vitamin D deficiency **(A)**, pooled odds ratio of vitamin D deficiency **(B)**, and pooled unstandardized mean difference of vitamin D levels **(C)** in androgenetic alopecia.

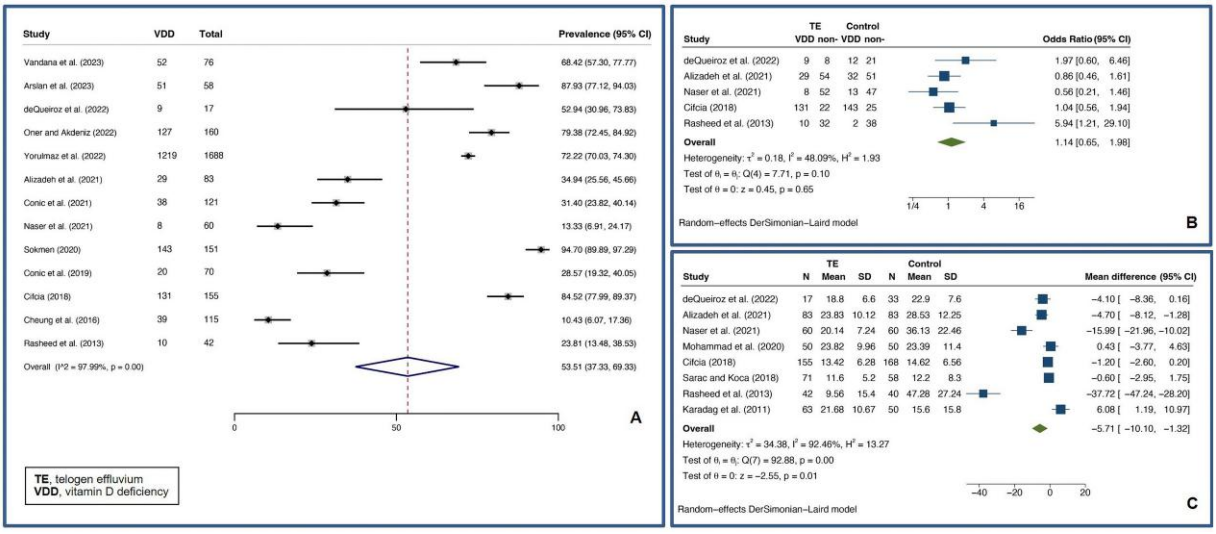

FIGURE 5
Forest plots for the pooled prevalence of vitamin D deficiency **(A)**, pooled odds ratio of vitamin D deficiency **(B)**, and pooled unstandardized mean difference of vitamin D levels **(C)** in telogen effluvium.

and TE patients also exhibited lower vitamin D levels compared to controls. Geographical factors exerted an influence, as the prevalence of VDD and the reduction in vitamin D levels were more pronounced in studies conducted in Eastern countries than in Western countries. Furthermore, younger patients aged 18 to 25 years exhibited a higher prevalence of VDD and more severe reductions in vitamin D levels

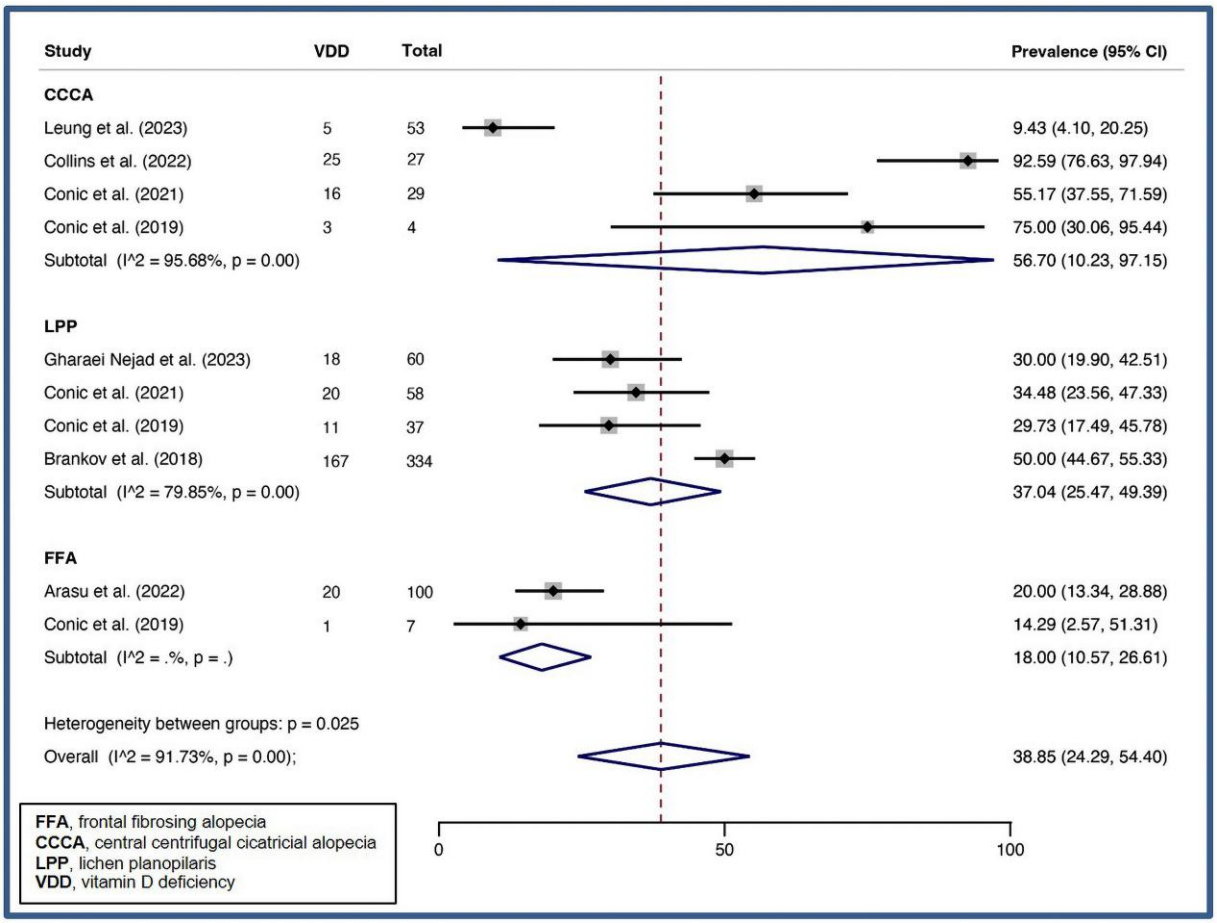

FIGURE 6
Forest plot for the pooled prevalence of vitamin D deficiency in primary lymphocytic scarring alopecia.

compared to older patients. The study also determined that patients with severe AA exhibited greater reductions in vitamin D levels compared to controls, though both severe and non-severe AA patients had comparable VDD prevalence. Overall, the findings suggest a significant relationship between vitamin D deficiency and certain types of alopecia, particularly AA and FPHL, underscoring the necessity for further research into the role of vitamin D in these conditions.

The role of vitamin D as an immunomodulator is particularly relevant in AA, in which autoimmune mechanisms are hypothesized to play a pivotal role. Vitamin D modulates the activity of cytotoxic T cells, regulatory T cells, and dendritic cells, all of which are involved in AA pathogenesis. Insufficient vitamin D levels may contribute to dysregulation of the immune response in AA, potentially resulting in an autoimmune attack on hair follicles (101).

Previously, there have been a few meta-analyses on VDD and AA. Similar to previous studies, we found that VDD is prevalent among AA, and compared to controls, AA had significantly higher odds of VDD and significantly lower vitamin D levels (102–104). In addition to updating the previous systematic review, we also pooled CC of AA disease severity and found a significant negative correlation between SALT scores and vitamin D levels, and severe AA was found to have a greater reduction of vitamin D level compared to control (*vs* non-severe AA). However, both severe and non-severe AA cohorts have similar odds of VDD. Currently, no cohort study has investigated the causal relationship between AA and VDD. Therefore, it is unknown whether VDD initiates AA pathogenesis or exacerbates AA conditions or whether AA causes VDD. A longitudinal study that investigates the connection between AA and VDD would provide evidence and strengthen the recommendation to screen AA patients for VDD.

A significantly stronger association between VDD and AA was observed in cohorts with AA patients with a disease duration of less than 1 year, suggesting that vitamin D status may play a crucial role in the early stages of AA development. Additionally, studies with age group of 18-to-25 years showed a higher risk of VDD, indicating that young adults with AA might be especially vulnerable to vitamin D deficiency. Interestingly, cohorts with a higher proportion of relapsed AA were found to have a higher risk of VDD, which aligns with the understanding of AA as an autoimmune disease (7). The relapsed state may represent compromised immune regulation, potentially exacerbated by low vitamin D levels (3, 7, 105–107). In contrast, cohorts with a lower proportion of females had lower vitamin D levels, hinting at possible gender-specific differences in vitamin D metabolism or AA pathogenesis. These findings underscore the complex interplay between vitamin D status and AA, highlighting the need for further research to elucidate the specific role of age, gender, and disease duration in this relationship. Such investigations could

TABLE 5 Pre-planned subgroup analyses based on country of research origin, age group, and alopecia severity.

	Alopecia areata			Androgenetic alopecia			Telogen effluvium		
Subgroup	No. of studies	Prevalence	I^2 (%)/*p*-value of Q test	No. of studies	Prevalence	I^2 (%)/*p*-value of Q test	No. of studies	Prevalence	I^2 (%)/*p*-value of Q test
Overall	34	51.94% (41.54–62.25%)	97.48/<0.01	16	47.27% (32.49–62.29%)	96.06/<0.01	13	53.51% (37.33–69.33%)	97.99/<0.01
Country									
Eastern	28	56.70% (43.04–69.87%)	97.88/<0.01	9	64.31% (48.41–78.81%)	92.10/<0.01	9	64.42% (49.17–78.33%)	97.10/<0.01
Western	6	31.36% (23.51–39.74%)	82.73/<0.01	7	25.54% (16.80–35.34%)	82.88/<0.01	4	27.56% (13.59–44.07%)	88.11/<0.01
Age									
Mean age 18–25 years	3	47.01% (1.32–96.78%)	–	3	70.69% (51.32–86.96%)	–	0	–	–
Mean age 25–60 years	29	54.67% (44.56–64.60%)	96.79/<0.01	11	42.07% (25.49–59.60%)	96.42/<0.01	9	56.00% (38.63–72.67%)	97.70/<0.01
Mean age > 60 years	1	5.56% (0.99–25.76%)	–	1	14.81% (5.92–32.48%)	–	1	28.57% (19.32–40.05%)	–
Unspecified	1	35.00% (18.12–56.71%)	–	1	68.00% (54.19–79.24%)	–	1	10.43% (6.07–17.36%)	–
Severity									
Severe cohorts	9	44.36% (19.70–70.54%)	98.01/<0.01	–	–	–	–	–	–
Non-severe cohorts	13	63.71% (47.43–78.58%)	95.25/<0.01						
Unspecified	12	44.64% (31.88–57.75%)	96.90/<0.01						

Subgroup	No. of studies	Odds ratio	I^2 (%)/*p*-value of Q test	No. of studies	Odds ratio	I^2 (%)/*p*-value of Q test	No. of studies	Odds ratio	I^2 (%)/*p*-value of Q test
Overall	24	2.84 (1.89–4.26)	84.29/<0.01	7	3.43 (0.95–12.35)	95.16/<0.01	5	1.14 (0.65–1.98)	48.09/0.10
Country									
Eastern	20	3.18 (2.04–4.97)	75.74/<0.01	5	5.65 (1.75–18.18)	79.96/<0.01	4	1.04 (0.56–1.94)	53.62/0.09
Western	4	1.78 (0.77–4.16)	91.62/<0.01	2	1.00 (0.18–5.59)	91.52/<0.01	1	1.97 (0.60–6.46)	–
Age									
Mean age 18–25 years	2	6.65 (1.22–36.35)	90.06/<0.01	1	13.50 (4.22–43.21)	–	0	–	–
Mean age 25–60 years	22	2.57 (1.70–3.88)	82.36/<0.01	6	2.73 (0.74–10.15)	93.99/<0.01	5	1.14 (0.65–1.98)	48.09/0.10
Severity									
Severe cohorts	8	3.29 (1.30–8.34)	85.53/<0.01	–	–	–	–	–	–
Non-severe cohorts	11	3.58 (2.20–5.82)	60.92/<0.01						
Unspecified	5	1.62 (0.71–3.71)	91.62/<0.01						

(Continued)

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TABLE 5 (Continued)

Subgroup	No. of studies	Mean difference	I^2 (%)/*p*-value of Q test	No. of studies	Mean difference	I^2 (%)/*p*-value of Q test	No. of studies	Mean difference	I^2 (%)/*p*-value of Q test
Overall	33	−8.20 (−10.28 – −6.12)	74.25/<0.01	15	−6.39 (−9.81 – −2.97)	88.56/<0.01	8	−5.71 (−10.10 – −1.32)	92.46/<0.01
Country									
Eastern	30	−8.24 (−10.02 – −6.46)	58.11/<0.01	12	−8.13 (−11.97 – −4.29)	87.64/<0.01	7	−6.07 (−11.02 – −1.13)	93.47/<0.01
Western	3	−6.67 (−23.12–9.77)	85.29/<0.01	3	−0.52 (−8.58–7.54)	84.55/<0.01	1	−4.10 (−8.36–0.16)	–
Age									
Mean age 18–25 years	5	−8.98 (−12.23 – −5.73)	0.00/1.00	1	−9.24 (−11.28 – −7.20)	–	0	–	–
Mean age 25–60 years	27	−8.07 (−10.44 – −5.70)	78.12/<0.01	12	−5.81 (−9.49 – −2.13)	90.34/<0.01	7	−6.74 (−11.69 – −1.80)	93.44/<0.01
Mean age > 60 years	0	–	–	0	–	–	0	–	–
Unspecified	1	−12.43 (−18.47 – −6.39)	–	2	−10.37 (−18.94 – −1.80)	88.56%/0.65	1	0.43 (−3.77–4.63)	–
Severity									
Severe cohorts	8	−10.65 (−15.23 – −6.07)	44.83/0.08	–	–	–	–	–	–
Non-severe cohorts	17	−8.17 (−9.97 – −6.37)	15.12/0.28						
Unspecified	8	−5.92 (−10.36 – −1.47)	89.79/<0.01						

provide valuable insights into the pathophysiology of AA and potentially inform more targeted therapeutic approaches.

As for pediatric AA, current evidence indicates that the prevalence and likelihood of VDD are lower than in the adult population, with non-statistically significant odds of having VDD compared to pediatric controls. Current evidence is relatively limited, and additional well-controlled studies are required to clarify the significance of VDD in pediatric AA.

To our knowledge, this is the first systematic review and meta-analysis to investigate the association between VDD and non-AA hair loss disorders, specifically AGA, TE, and scarring alopecia. Both TE and AGA were found to have a VDD prevalence of approximately 50% and significantly lower vitamin D levels than controls but did not have an increased risk of VDD compared to controls. However, the likelihood of VDD in FPHL patients is statistically significantly higher than in controls. Vitamin D levels in FPHL cohorts are significantly lower than in controls compared to AGA in general. Although MAGA data is limited, we hypothesize that gender plays a significant role in VDD and AGA (108, 109).

Our study found that in Eastern countries, the prevalence of vitamin D deficiency, the likelihood of VDD, and the degree of lower vitamin D levels among AA, AGA, and TE were significantly higher than in Western nations. European Caucasians have a lower prevalence of VDD than non-white individuals (110). In addition to differences in skin pigmentation, the use of sunscreen and latitude are also significant factors that could cause VDD by reducing vitamin D synthesis (111–113). Melanin in darker skin tones can interfere with vitamin D synthesis, while widespread sunscreen use and higher latitudes with less direct sunlight can reduce vitamin D production (114). Moreover, because these factors are rarely matched but could significantly influence the results, additional studies matching sunscreen use and FST are required.

Due to the limited number of case–control studies, the relationship between VDD and scarring alopecia remains poorly understood. However, our analysis suggests that the prevalence of VDD may vary among different types of PCA. Based on available data, certain PCA diseases may have a higher VDD prevalence than others. For instance, CCCA has a VDD prevalence of 57%, whereas FFA has a VDD prevalence of 18%. However, the higher prevalence of VDD among CCCA patients may not be due to the disease itself but rather their skin pigmentation, as it almost exclusively affects females with FST V-VI, which are known to be associated with a higher risk of VDD due to reduced vitamin D synthesis in darker skin (111, 115). To establish a more definitive association between scarring alopecia and VDD, and to better understand the role of confounding factors such as skin type, additional well-designed controlled studies are required.

The results of our study should be interpreted with caution due to the highly heterogeneous study population and setting of the included studies, as well as the fact that serum vitamin D levels can be affected by a variety of factors, such as geographic characteristics, ethnicity, and skin tone (111). Subgroup analyses were conducted to explore potential sources of heterogeneity; however, for most alopecia types, significant sources of heterogeneity were not identified, suggesting that residual heterogeneity may be attributed to unmeasured variables or other contextual factors not captured in the included studies. Future research should focus on more detailed reporting and examination

of factors contributing to heterogeneity. Also, publication bias exists in the analyses of the pooled UMD in vitamin D levels between AGA and controls, which necessitates caution when interpreting our results.

The study has several strengths and limitations. One of the strengths is its comprehensive approach, utilizing a systematic review and meta-analysis to pool data from a wide range of studies. The study also follows rigorous methodological standards, including the registration of the protocol in PROSPERO, adherence to PRISMA guidelines, and the use of validated tools such as the NOS for quality assessment, which contributes to the robustness of the conclusions. However, the study has some limitations. The heterogeneity of the included studies suggests considerable variability in study populations, settings, and methodologies, which could affect the reliability of the pooled estimates. Moreover, the study's reliance on observational data limits the ability to infer causality. Finally, the limited number of studies on scarring alopecia and pediatric populations restricts the generalizability of the findings to these subgroups.

5 Conclusion

Even though VDD is prevalent among alopecia patients, the likelihood of VDD and decreased vitamin D levels compared to the control population was statistically significant only in adult AA and FPHL. Adult AA disease severity was found to be significantly negatively correlated with vitamin D levels, with severe AA cohorts showing a higher reduction of vitamin D levels compared to controls; however, severe and non-severe AA cohorts appear to have comparable VDD prevalence and VDD likelihood compared to controls. Cohorts with less than one year of AA duration and a higher proportion of relapsed AA were found to have a higher risk of VDD, while cohorts with a lower proportion of females had lower vitamin D levels. Studies conducted in Eastern nations appear to report a higher VDD prevalence, VDD likelihood, and vitamin D reduction than studies conducted in Western nations. Evidence is still lacking for MAGA, scarring alopecia, and pediatric AA, highlighting the need for further research in these areas. It is important to acknowledge the limitations of this study, including the heterogeneity of the included studies. Given these results, clinicians should consider routine screening for VDD in patients with severe AA or FPHL, particularly in Eastern countries or in patients with recent onset or relapsed AA. Early detection and potential correction of vitamin D deficiency could play a role in managing the severity and progression of alopecia. Future studies should focus on addressing the gaps in our understanding of the role of vitamin D in alopecia, particularly MAGA, scarring alopecia, and pediatric AA, as well as investigating the potential benefits of vitamin D supplementation in alopecia management.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

TY: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. KaT: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – review & editing. KuT: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing. PS: Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1479337/full#supplementary-material

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Comparative analysis of COVID-19 responses in Japan and Africa: diet, phytochemicals, vitamin D, and gut microbiota in reducing mortality—A systematic review and meta-analysis

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Background: As the novel coronavirus disease 2019 (COVID-19) pandemic subsides, the clinical sequelae are becoming more problematic. Interestingly, the statistical data indicate that Africa has experienced the lowest number of cases and deaths, with an unexpected phenomenon where the number of deaths from COVID-19 has not increased significantly. Several studies have investigated the relationship between diet and coronavirus. However, no systematic review/meta-analysis has conclusively linked diet (phytochemicals and vitamin D) and the gut microbiota in the context of COVID-19.

Methods: This study examined the responses to COVID-19 in Japan and Africa, formulating the following hypotheses: (1) a healthy diet is effective against COVID-19, (2) blood vitamin D levels are associated with COVID-19 mortality, and (3) COVID-19 is associated with the gut microbiota. To investigate these hypotheses, a keyword search and meta-analysis were conducted using PubMed, and each hypothesis was tested.

Results: This study found that a healthy diet, particularly rich in phytochemicals such as polyphenols and flavonoids, is effective against COVID-19. An association was detected between blood vitamin D levels and COVID-19 mortality. The gut microbiota was linked to COVID-19 and its amelioration. These findings may have significant implications for not only understanding COVID-19 but also future prevention of pneumonia.

KEYWORDS

COVID-19, phytochemicals, polyphenols, flavonoids, vitamin D, gut microbiota, Japan, Africa

1 Introduction

The COVID-19 pandemic has caused a global crisis, reminiscent of the Spanish flu of 1918, with severe consequences for the global economy. Pneumonia caused by coronaviruses is a zoonosis, and humans have experienced the emergence of three highly pathogenic CoV species over the past two decades: severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, and SARS-CoV-2 (1). Its strong infectivity has been verified through transmission from humans to cats, which may have served as intermediate hosts for the virus (2). Regarding the COVID-19 vaccine, some ecological studies have shown regional disparities in immunization coverage in the USA (3). There is concern regarding low vaccination rates despite the greater risk of infection in non-Hispanic Black and Hispanic populations. Data provided by the WHO as of June 17, 2024, showed that the COVID-19 deaths in Africa, the Americas, Europe, the Eastern Mediterranean region, and Asia (Western Pacific and South-East Asia) numbered 175,510 (2%), 3,020,756 (43%), 2,272,390 (32%), 351,975 (5%), and 1,229,712 (17%), respectively (4). Africa had the lowest proportion of cumulative deaths worldwide at 2%, accounting for 9,579,844 cumulative cases and only 1% of the global total.

Contrary to expectations, the number of COVID-19 deaths did not increase significantly in Africa despite the high rates of HIV, malaria, and other infectious diseases and the lack of developed healthcare systems. In contrast, in many developed countries in Europe and the USA, which have large elderly populations, COVID-19 resulted in high mortality rates, especially among the elderly and those with underlying medical conditions. Elderly individuals are more susceptible to pneumonia, with underlying conditions such as diabetes and obesity, which are metabolic conditions included in lifestyle-related diseases, and a weakened immune system due to various diseases. These susceptible populations prioritized vaccination and other preventive measures.

In Africa, an interesting phenomenon was observed: the number of deaths due to COVID-19 did not increase, as expected. However, this anomaly requires further investigation. One possible reason for this is the demographic structure of Africa, which has an overwhelmingly large number of children and a relatively small number of elderly individuals, who showed higher mortality rates from COVID-19. The median age on the African continent is 18.8 years, compared to the world average of 30.7 years and 49.5 years in Japan (5). In children, the innate immune response that eliminates the virus may be more effective than the speed at which the virus mutates (6, 7).

Another explanation is that Japan's low mortality rate from COVID-19 compared to that in Western countries comes from its status as the country with the longest life expectancy in the world (8). Reports have shown that the nutritional situation in Africa has been adversely affected by the COVID-19 pandemic, particularly among children (9). In addition to economic development, a well-known factor contributing to Japan's longevity is the healthiness of the Japanese diet. The Japanese diet, similar to the Mediterranean diet (10), has also been considered healthy, particularly around 1975, which is considered healthier than the current Japanese diet (11–13). Compared to the USA and other countries, Japan has lower rates of obesity and lifestyle-related diseases, including metabolic diseases, which are believed to be associated with the longevity of its population. The typical Western diet is high in energy density, leading to underlying and lifestyle-related diseases and impaired immunity due to chronic inflammation, which have been identified as risk factors for COVID-19 (14). This study investigated the effects of polyphenols, a class of phytochemicals abundant in the healthy diets of the Mediterranean region and Japan, on COVID-19.

It is also well-established that vitamin D deficiency is associated with a range of diseases, including those that impair immunity. A systematic review and meta-analysis conducted in Italy, a country severely affected by COVID-19, revealed a clear association between vitamin D deficiency and COVID-19 mortality (15). Blood vitamin D levels are categorized as follows: < 20 ng/mL deficient, 20–30 ng/mL, insufficient; and > 30 ng/mL, sufficient. A report showed the following blood vitamin D (25-OH-D) levels (ng/mL) in European countries: France 24.0, Germany 20.0, Italy 20.0, the UK 19.0, and Spain 17.0 (16). In Japan, only 2% of individuals have sufficient blood vitamin D levels, with an average value of 15.5 ng/mL (17). In Africa, because of the strong direct sunlight, over 40% of people have blood vitamin D levels above 30 ng/mL, with a mean value of 27.1 ng/mL, the highest among the compared regions (18).

Finally, the relationship between the gut microbiota and COVID-19, which is significantly influenced by diet and varies with the disease, was also examined.

Based on the above, this study tested the following hypotheses by examining COVID-19 responses in Japan and Africa: (1) a healthy diet is effective against COVID-19, (2) blood vitamin D levels are associated with COVID-19 mortality, and (3) COVID-19 is associated with the gut microbiota. To test these hypotheses, we conducted a series of literature searches and summarized the findings of a systematic review and meta-analysis.

2 Materials and methods

2.1 Selection criteria, sources, and search strategy

This systematic review follows Cochrane guidelines and reports using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (19). Compliance with the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist is shown in the Supplementary Table S1. Articles with specific keywords in the title or abstract were selected for this study. The PubMed search engine was used for this systematic review and meta-analysis. The keywords used in this systematic review are: Japan, Africa, polyphenol, flavonoids, vitamin D, and gut microbiota. Polyphenols and flavonoids are major classes of phytochemicals. Four patterns of keyword searches have been shown in the results: Analysis Group A–D.

The protocol was registered in UMIN-CTR under the UMIN study ID: UMIN000054334.[1] Ethical approval was not required for this study, as all the data used are publicly available. The examined literature was peer-reviewed and written in English. The search for COVID-19-related articles covered the period from 2019 to the 25th of July 2024.

1 https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000062073

2.2 Selection procedure and exclusion criteria

Records were initially identified using a PubMed database search. Duplicate records were excluded from the systematic review. Two independent reviewers (KS and RT) assessed the titles, abstracts, and entire articles, including the results of the identified studies, and judged the inclusion and exclusion of any irrelevant reports. Disagreements regarding the inclusion of studies were resolved through discussions and consensus. If disagreements persisted, they were arbitrated by another reviewer. One example of an excluded study is changes in blood 25-OH-D concentration, bone markers, and physical performance due to vitamin D supplementation while COVID (COVID-19) lock down since this study might appear to meet the inclusion criteria, but these were excluded because they were not related to COVID-19 treatment (20). In some analysis groups, articles other than randomized clinical trials (RCTs) were also excluded. A summary of information derived from up-to-date studies on vitamin D was also used as background information.

2.3 Data items and data collection process

Data collection included the following elements: study characteristics (author, year of publication, title, and abstract), participants (selected from relevant RCTs in the context of COVID-19 or long-COVID-19) and keywords in each grouped analysis.

Analysis group A: A two-keyword search was conducted to summarize the COVID-19 responses in Japan and Africa. A keyword search (Japan), (COVID), and (vitamin D, polyphenol, flavonoids, or gut microbiota) yielded 100 results. Only one study was an RCT. Therefore, top 10 search results sorted according to "Best Match" and content related to COVID-19 were showed in the results. Only articles where the full text was available for free were used. Another keyword search, (Africa) and (COVID) and (vitamin D or polyphenol or flavonoids or gut microbiota) yielded 53 results, among which 40 had the full text available for free. Then, top 10 search results sorted by "Best Mach" and title related with COVID-19 were showed in the Tables 1, 2 in the results. Furthermore, two of the four RCTs in the search results as well.

Analysis Group B: For the blood 25-OH-D and COVID-19, search results for (COVID) and (vitamin D) were classified as enough (> 30 ng/mL), insufficient (20 to 30 ng/mL), and deficient (< 20 ng/mL) by the mean concentration of the intervention groups. Of the 1,893 results, 48 were RCTs and were further filtered out based on the availability of free full texts, resulting in 43 articles. From these results, 20 articles containing values of blood 25-OH-D3 with mean ± SD or median were summarized in the table. In addition, a meta-analysis was conducted using the data contained in these articles. Furthermore,

TABLE 1 Analysis Group A: Top 10 articles sorted by best match in PubMed - Japan, COVID and (polyphenol or flavonoids or vitamin D or gut microbiota) (As of 25th of July).

Research	Main findings	First author, year, references
Fundamental immuno modulatory effects of vitamin D in COVID-19 pandemic (Review)	Anti-inflammatory effects of 1α-25-(OH)2-D through VRDs and 1α-hydroxylase expressed on the immune cells in COVID.	Ao et al. (2021) (23)
Fecal shotgun metagenomic sequencing and metabolomics in SARS-CoV-2 infected 112 hospitalized patients and 112 control subjects.	Discovery of correlations between oral microbes, short-chain fatty acid producers, and intestinal metabolites associated with COVID-related microbes, and association with inflammatory cytokine dynamics.	Nagata et al. (2023) (24)
Summary of intestinal barrier (mechanical, chemical, microbial, and immune barrier) disruption mechanism by SARS-CoV-2 (Review).	Presentation of disruptive mechanisms of intestinal integrity of mechanical, chemical, microbial, and immune barriers by SARS-CoV-2 infection in COVID-19 including gastrointestinal symptoms.	Xue et al. (2023) (25)
Association between trypsin self-degradation by Paraprevotella strains and severity of diarrhea in COVID-19 patients.	Colonization of *Paraprevotella* strains inhibits the mouse coronavirus lethal infection through the inhibition of trypsin and trypsin-like protease dependent host cell invasion.	Li et al. (2022) (26)
Effects of tea catechins in SARS-CoV-2 Omicron subvariant.	Green tea, Matcha and black tea polyphenols effectively inactivate SARS-CoV-2 Omicron subvariant.	Shin-Ya et al. (2023) (27)
Research in oral fluid-based biomarkers in the detection of SARS-CoV-2 in saliva.	Oral and periodontal disease biosensor and lab-on-a-chip biomarkers detect SARS-CoV-2 in saliva.	Steigmann et al. (2020) (121)
Anti-viral (COVID) and anti-inflammatory effects of phytochemical-containing essential oil (Review).	Olfactory training with phytochemicals contained in lemon, rose, clove, and eucalyptus essential oil improve olfactory functions.	Koyama et al. (122)
Changes of microbiome in COVID-19 (Clinical study).	Gut microbiota diversity increase after the recovery from COVID-19, protective effects of Bacteroids in severe SARS-CoV-2 infection.	Babszky et al. (123)
Improvement of oxidative stress in COVID-19 outpatients by vitamin D supplementation.	Comparison between COVID patients and healthy subjects in anti-oxidative and anti-inflammatory effects, vitamin D supplementation suppress SOD, GPx, and TAC levels in COVID patients.	Golabi et al. (2022) (124)
High body temperature induced by the influenza A virus and SARS-CoV-2 infection increases gut microbiota-dependent host resistance.	Physiological role of fever in host resistance to viral infection, upregulation of immune response. Gut microbiota produced deoxycholic acid (DCA) and TGR5 signaling pathways suppress the viral replication and neutrophil dependent tissue damage.	Nagai et al. (2023) (125)

TABLE 2 Analysis Group A: Top 10 articles sorted by best match in PubMed - Africa, COVID and (polyphenol or flavonoids or vitamin D or gut microbiota) (As of 25th of July).

Research	Main findings	First author, year, references
The early age and plant-based diet hypotheses of low SARS-CoV-2 infection and the COVID-19 pandemic in sub-Saharan Africa (review)	Higher metabolic syndrome ratio is associated with higher risk of COVID infection. Africa has the lowest ration of metabolic syndrome. Plant-based diet includes whole grain, legumes, vegetables, potatoes, pumpkins, banana, moringa leaves, and reduced meat consumption. Plant based diet provides unique gut microbiome and extended survival ratio.	Losso et al. (2021) (28)
Acute and subacute oral toxicity characterization and safety assessment of Madagascar's anti-COVID herbal tea in animal models.	Herb tea consists of *Artemisia annua* (62%), and other plants (38%) was confirmed safe in mice.	Aina et al. (2023) (126)
Comparative analysis of Beninese and Chinese herbal medicine in COVID-19 treatment.	Identified herbal medicine used in Benin compared with Chinese herbal medicine, efficacy was vitrified *in vitro*. *Citrus aurantiifolia* (13.18%), *Momordica charantiantia* (7.75%), *Ocimum gratissimum* (7.36%), *Crateva adansonii* (6.59%), *Azadirachta indica* (5.81%), *Zanthoxylum zanthoxyloides* (5.42%) were the most used.	Houeze et al. (2023) (29)
Efficacy of propolis in SARS-COV-2 virus: anti-viral effects and molecular simulation (Review)	Propolis polyphenol reduce the replication of virus and beneficial for the treatment of SARS-CoV-2 infected patients.	Ghosh et al. (2022) (127)
Effects of resveratrol in COVID-19 (Review)	Resveratrol in safe, affordable, and available adjuvant treatments.	van Brummelen et al. (2022) (30)
Randomized trials, meta-epidemiological cohort study of hydroxychloroquine, corticosteroids, and vitamin D in COVID-19 (meta-analysis).	Hydroxychloroquine, corticosteroids, and vitamin D as a treatment of COVID-19, less than one third of registered trials made their results public.	Fincham et al. (2024) (31)
Research of effective molecular against coronavirus protease using flavonoids.	Inhibition of SARS-CoV-2 main protease (Mpro) by quercetin-3-O-Neohesperidoside is the candidate of COVID-19 treatment.	Fadaka et al. (2020) (128)
Research of COVID-19 severity and vitamin D levels.	COVID-19 patients in 82% were vitamin D deficiency or insufficient. Patients in vitamin D deficiency were higher risk of COVID-19 infection.	Kalichuran et al. (2022) (32)
Verification of cytotoxic and anti-viral effects of *Bersama abyssinica* extract in SARS-CoV-2 delta variant.	*B. abyssinica* water extract used in COVID-19 treatment had significant antiviral effect in SARS-CoV-2 but no cytotoxic in Vero E6 cells.	Zekeya et al. (2022) (129)
Effects of probiotics in the war against COVID virus (Review).	Intestinal probiotics, lactic acid bacteria (LAB) and *Bifidobacterium* spp. were decreased in COVID-19 patients. Explored the potential of probiotic bacteria and their metabolites to intervene with the process of virus infection.	Tiwari et al. (2020) (130)

a meta-analysis of enough blood 25-OH-D levels (> 30 ng/mL) in the intervention group was conducted.

Analysis group C: Combined keywords (COVID and polyphenol) yielded 410 results, (COVID and flavonoids) yielded 818 results, (COVID and vitamin D) yielded 1,893 results, and (COVID and gut microbiota) yielded 1,012 results. A total of 4,142 studies appeared in the PubMed search. A combined search of (COVID) and (polyphenols, flavonoids, vitamin D, and gut microbiota) showed 3,949 results. Therefore, 193 duplicate results were excluded. As only RCTs were considered, 3,875 articles were excluded, leaving 74 eligible papers. Of these, 33 studies were excluded because they did not meet the exclusion criteria described above. Finally, 41 studies were included in this systematic review. The screening process is described in detail in the PRISMA flowchart (Figure 1). A meta-analysis was performed on the search results for (COVID and vitamin D) in the analysis group C.

Analysis group D: Existing articles within the 5 years when COVID-19 related articles were available were searched for in PubMed. A combination of keywords (polyphenols or flavonoids) and (gut microbiota) yielded 2,979 results. Within the last 5 years when COVID-19 was prevalent, 2,225 search results were obtained. Of these, 48 were RCTs. When narrowed down to the past year, 573 papers and 9 RCTs were identified, fitting the study's objectives, resulting in seven papers being included. A PubMed search for (vitamin D) and (gut microbiota) yielded 532 results, with 389 results published in the last 5 years. Among these, 16 were RCTs, and five were fit-for-purpose articles. A PubMed search for (polyphenols, flavonoids) and (vitamin D) yielded 622 results, of which 188 were obtained in the last 5 years. Of these, 11 were RCTs, and six were fit-for-purpose articles.

2.4 Outcome

To verify the three hypotheses: (1) a healthy diet is effective against COVID-19, (2) blood vitamin D levels are associated with COVID-19 mortality, and (3) COVID-19 and gut microbiota are associated, search results from Analysis Groups A, B, C, and D have been summarized into tables in the results section.

2.5 Statistical analysis

A series of meta-analyses of the articles corrected in Analysis Groups B (COVID and vitamin D) and C were conducted (articles in Tables 3–5, 8). Meta-analysis has been performed by EZR [R 4.4.1 binary

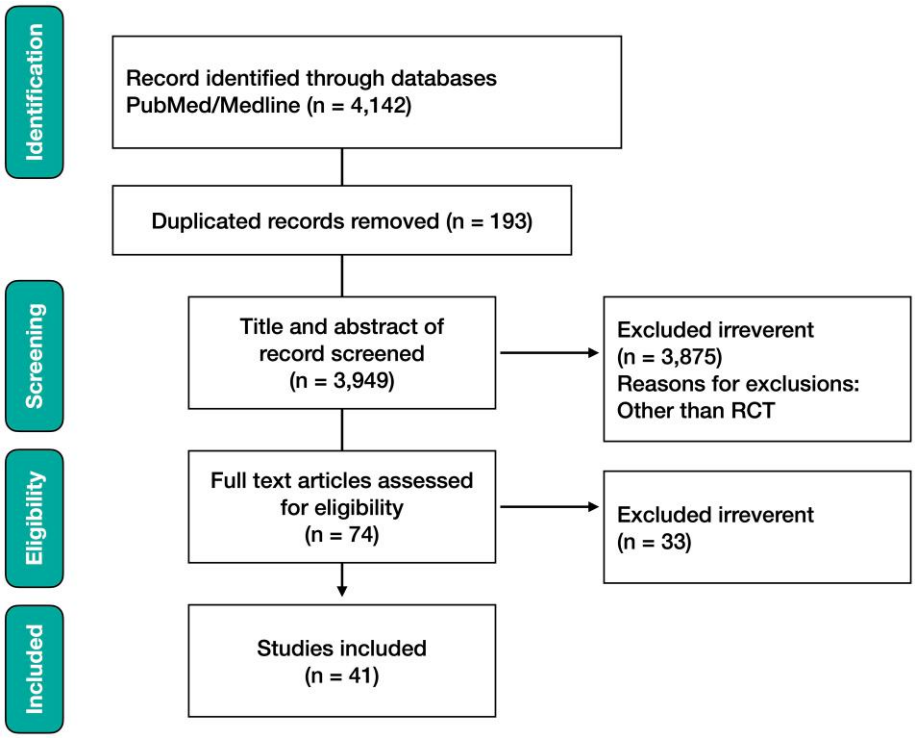

FIGURE 1
PRISMA flowchart of this systematic review: Analysis Group A. PubMed search with (COVID) and each keyword (polyphenol or flavonoids), (vitamin D), and (gut microbiota) showed 4,142 results. Combined search of (COVID) and (polyphenol or flavonoids or vitamin d or gut microbiota) showed 3,949 results. Duplicated 193 results were excluded. Then, 3,875 results other than randomized clinical trials (RCTs) were excluded and only 74 RCTs were considered further analysis. Of these, 33 studies were excluded based on the exclusion criteria because they did not meet the purpose of the analysis. Finally, 41 manuscripts were exploited in this systematic review (as of 25th of July 2024).

for macOS 11 (Big Sur)] software downloaded from "The Comprehensive R Archive Network" webpage.[2] References including median values were converted to mean ± SD from the first and third tetrad counts. Meta-analysis for means were conducted to analyze the data sets including mean ± SD and total number of samples. The standard mean difference (SMD) with 95% confidence interval (CI) was reported for dichotomous outcomes. A meta-analysis for proportions was conducted to analyze the datasets, including events and the total number of samples. Odds ratios (ORs) with 95% confidence intervals (CI) were reported for dichotomous outcomes. This study performed both fixed-and random-effects modeling. $p < 0.01$ was considered statistically significant.

3 Results

3.1 Comparison of Japan and Africa's population

Figure 2 shows the population pyramids of Japan, Africa, and the rest of the world. The largest population group in Japan peaked in the age group of 50–54 (9,510,374 people), followed by the age group of 70–74 (8,218,437 people). These were the second and first baby boomers, respectively (21). The Japanese population is a typical example of an aged society that is common in developed countries, with people older than 65 years comprising a quarter of the population. Surprisingly, the proportion of women older than 100 years was 0.1% in Japan. In contrast, Africa had a typical juvenile population pyramid; as the population became younger, the number of people in Africa increased. However, the world population showed a bell-shaped pyramid. Since COVID-19 vaccination efforts were prioritized for the elderly and people with underlying diseases in Japan, an African population with an enormous number of children was considered one of the reasons for Africa's low number of COVID-19 deaths.

3.2 Analysis group A: Summary of COVID response in Japan and Africa

3.2.1 Summary of COVID response in Japan

Only one RCT was found after searching for Japanese COVID-19 responses to oral vaccination against Tuberculosis (22). Table 1 summarizes the 10 articles on Japan's response to COVID-19. These articles were publication types other than RCTs, and were conducted in Japan or by authors belonging to Japanese research institutions. They include a review of the effects of vitamin D against COVID-19 through the vitamin D receptors (VDRs) expressed on the surface of immune cells (23); a metabolomics research related to COVID (24); a review of the mechanism of severe gastrointestinal conditions in COVID-19 (25); association between the enhancement of trypsin self-degradation by *Paraprevotella* colonization and severity of diarrhea in

2 https://cran.r-project.org/

TABLE 3 Analysis Group B: Association between COVID-19 and blood 25-OH-D3 levels; mean value of the Intervention group showed enough level (>30ng/mL) RCT.

First author, year, references	Subjects numbers		Mean 25-OH-D (ng/mL)		SD	
	Intervention	Control	Intervention	Control	Intervention	Control
Mariani et al. (2022) (131)	115	103	102.00	30.00	34.81	2.59
Bishop et al. (2023) (132)	65	69	82.00	37.00	4.00	1.00
Fernandes et al. (2022) (133)	101	99	44.60	19.80	14.70	10.50
Murai et al. (2021) (134)	120	120	44.40	19.80	15.00	10.50
Jolliffe et al. (2022) (135)	956	908	42.16	21.44	9.40	10.08
Mahjoub et al. (2024) (136)	34	24	42.00	19.30	13.70	8.50
Haas et al. (2024) (137)	17	41	35.36	37.20	11.04	12.24
Karonova et al. (2022) (50)	45	46	32.90	19.30	9.85	9.70
Murai et al. (2021) (138)	16	16	31.70	7.80	12.30	1.70
Caballero-García et al. (2021) (139)	15	15	31.30	19.40	1.40	2.30

TABLE 4 Analysis Group B: Association between COVID-19 and blood 25-OH-D3 levels; mean value of the Intervention group was sufficient level (20 to 30ng/mL) RCT.

First author, year, references	Subjects numbers		Mean 25-OH-D (ng/mL)		SD	
	Intervention	Control	Intervention	Control	Intervention	Control
Brunvoll et al. (2022) (140)	278	17,323	29.64	25.12	8.27	9.90
Torres et al. (2022) (52)	41	44	29.22	19.11	6.89	8.69
Cannata-Andía et al. (2022) (141)	274	269	29.00	16.40	10.89	7.78
Cesur et al. (2023) (48)	16	17	27.18	14.78	12.08	10.75
Villasis-Keever et al. (2022) (142)	94	98	26.10	19.30	7.41	8.52
Sabico et al. (2021) (53)	36	33	25.00	23.96	1.36	1.56
Karonova et al. (2022) (143)	56	54	22.80	10.60	7.04	4.81
Annweiler et al. (2022) (47)	127	127	21.20	17.20	12.15	17.19
Bychinin et al. (2022) (51)	55	55	20.60	9.60	9.63	11.11

TABLE 5 Analysis Group B: Association between COVID-19 and blood 25-OH-D3 levels; mean value of the Intervention group was insufficient level (<20ng/mL) RCT.

First author, year, references	Subjects numbers		Mean 25-OH-D (ng/mL)		SD	
	Intervention	Control	Intervention	Control	Intervention	Control
De Niet et al. (2022) (144)	50	50	17.87	16.87	0.15	9.48

patients infected with SARS-CoV-2 (26), and effects of green tea catechins on Omicron variants (27).

3.2.2 Summary of COVID response in Africa

Table 2 summarizes the 10 articles associated with COVID-19 responses in Africa. A review article on plant-based diets in sub-Saharan Africa reasoned that a low metabolic syndrome ratio is associated with a low COVID-19 risk in Africa (28). Others include the identification of herbal plants administrated to COVID patients in Benin (29); resveratrol as a safe, affordable, and available adjuvant treatment (30); meta-analysis of corticosteroids, hydroxychloroquine, and vitamin D as treatments for COVID-19 (31); and a report on high COVID-19 risks in patients with vitamin D deficiency (32). Only four RCTs were found in African research; however, one was associated with tuberculosis prevention (33) and the others were about egg consumption to improve diet (34).

3.3 Analysis Group B: Association between COVID-19 and blood 25-OH-D3 levels; deficient, insufficient, and enough amount

Of the 43 RCTs on COVID and vitamin D, 20 articles held mean ± SD or median values were summarized and divided by the serum vitamin D levels of the intervention group into three groups: enough (> 30 ng/mL) amount (Table 3), insufficient (20 to 30 ng/mL) (Table 4), and deficient (< 20 ng/mL) (Table 5). The mean value in the enough amount group varied from 31.3 to 102 (ng/mL), that in the

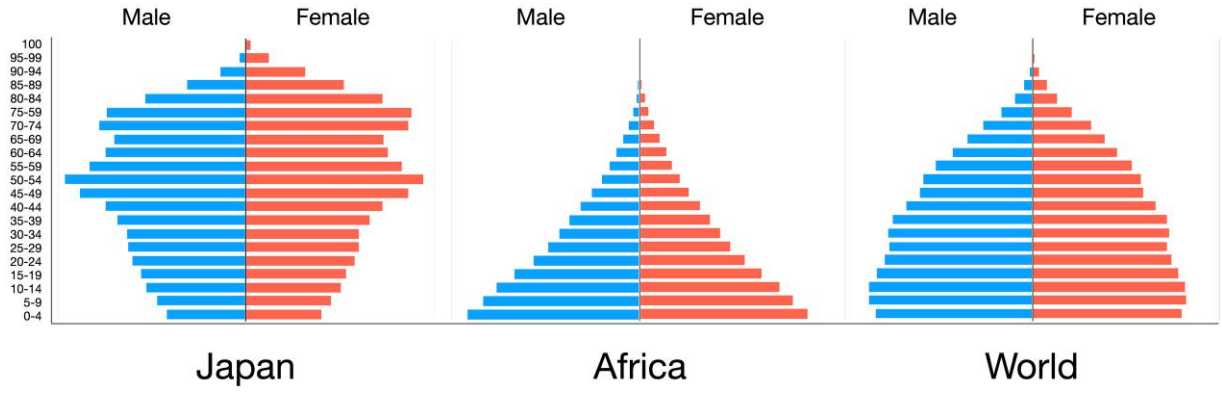

FIGURE 2
Population pyramid of Japan, Africa, and the World in 2024. Population pyramid of Japan was a typical shape of an aged society with large numbers of baby boomer seen in developed countries. Population groups peaked around 45–49 and 70–74 years old. Africa had a typical juvenile-formed population pyramid. Population pyramid of the world total was bell-shaped. One reason of Africa's low death ratio of COVID-19 seemed to come from this very young population distribution.

insufficient group varied from 20.8 to 29.64, and the deficiency group had one result with a level of 17.87.

Then, meta-analysis was performed only on studies that met both conditions: blood vitamin D levels of 20 (ng/mL) or higher in the intervention group and 20 or lower in the control group. A meta-analysis performed on articles in Tables 3–5, and the results of adequate heterogeneity ($I^2 = 72\%$) are shown in Figure 3A. Another meta-analysis with enough (> 30 ng/mL) blood 25-OH-D levels in the intervention groups are shown in Figure 3B.

3.4 Analysis group C: Nutrients and gut microbiota against COVID-19

3.4.1 Validation of the effectiveness of a healthy diet against COVID-19

This systematic review shows the relevance of polyphenols and flavonoids, two of the most common phytochemicals associated with a healthy diet, in relation to COVID-19. Table 6 summarizes the four RCTs on COVID-19 and polyphenols, and Table 7 summarizes the eight RCTs on COVID-19 and flavonoids. Flavonoids are a typical component of polyphenols. The polyphenol curcumin promotes recovery from COVID-19 by improving blood oxygen saturation (35). Additionally, daily consumption of high-polyphenol olive oil was found to significantly reduce treatment duration (36). Two RCTs involving resveratrol, a polyphenol that was first highlighted for its presence in red wine, were included. The first study demonstrated its effectiveness against respiratory infections, including COVID-19 (37), and the second showed that resveratrol reduced the expression of ACE2, a receptor for COVID-19, in the adipose tissue (38). The flavonoid quercetin was found to reduce the expression of markers associated with COVID-19 severity when combined with anti-viral drugs used to treat COVID-19, such as remdesivir and favipiravir. This included effectively lowering levels of serum alkaline phosphatase (ALP), quantitative C-reactive protein (q-CRT), and lactate dehydrogenase (LDH) (39). Silymarin also reduced alanine aminotransferase levels (40). Several studies in Italy reported that luteolin is effective against olfactory abnormalities, one of the symptoms of COVID (41–45). Additionally, the use of gargles containing the bioflavonoids β-cyclodextrin and Citrox (CDCM) was shown to reduce coronavirus presence (46). Phytochemicals are considered the seventh most abundant nutrient and have been shown to be effective against COVID-19.

3.4.2 Validation of the association between blood vitamin D levels and COVID-19 mortality

Table 8 summarizes the relevant RCTs on COVID and vitamin D. Among the 41 studies, 20 were found to be relevant (1 μg = 40 IU). The studies mainly involved the administration of high concentrations of oral vitamin D3, active calcitriol (1α,25-(OH)2-D3), alfacalcidol, and calcidiol (25-OH-D3), which is used to measure vitamin D levels in the blood. The conversion of 25-OH-D3 to calcitriol is facilitated by enzymes in the kidneys or immune cells. High-dose vitamin D3 has been reported to reduce mortality in COVID-19 patients (47) increase vaccine antibody production (48), suppress cytokine storms (49), increase blood 25-OH-D3 levels (50) and lymphocyte counts (51), shorten hospital stays (52), and recovery time (53), reduce healthcare utilization due to COVID-19 (54). There is substantial evidence that vitamin D supplementation is effective during the COVID-19 pandemic and helps improve sequelae such as loss of taste (55).

However, it is important to note that long-term intake of higher-than-necessary doses of vitamin D, especially with calcium, should be avoided as it can cause vitamin D toxicity. Therefore, vitamin D should be considered an immune-enhancing nutrient rather than a therapeutic agent.

In addition, a meta-analysis was performed on the 20 articles shown in Table 8, and the quantitative analysis is summarized in Figure 4. Blood 25-OH-D3 levels were found in 12 articles supplemented with vitamin D3 (10 articles) and 25-OH-D3 (two articles). Five articles showing only medians were converted into mean ± SD. The values of the five articles indicating adequate heterogeneity ($I^2 = 73\%$) are shown in a forest blot (Figure 4A). Similarly, analysis of two articles showing the length of hospitalization period described in mean ± SD and total number were shown (Figure 4B). Three articles regarding COVID-19 cases (Figure 4C) and two articles regarding COVID-19 deaths

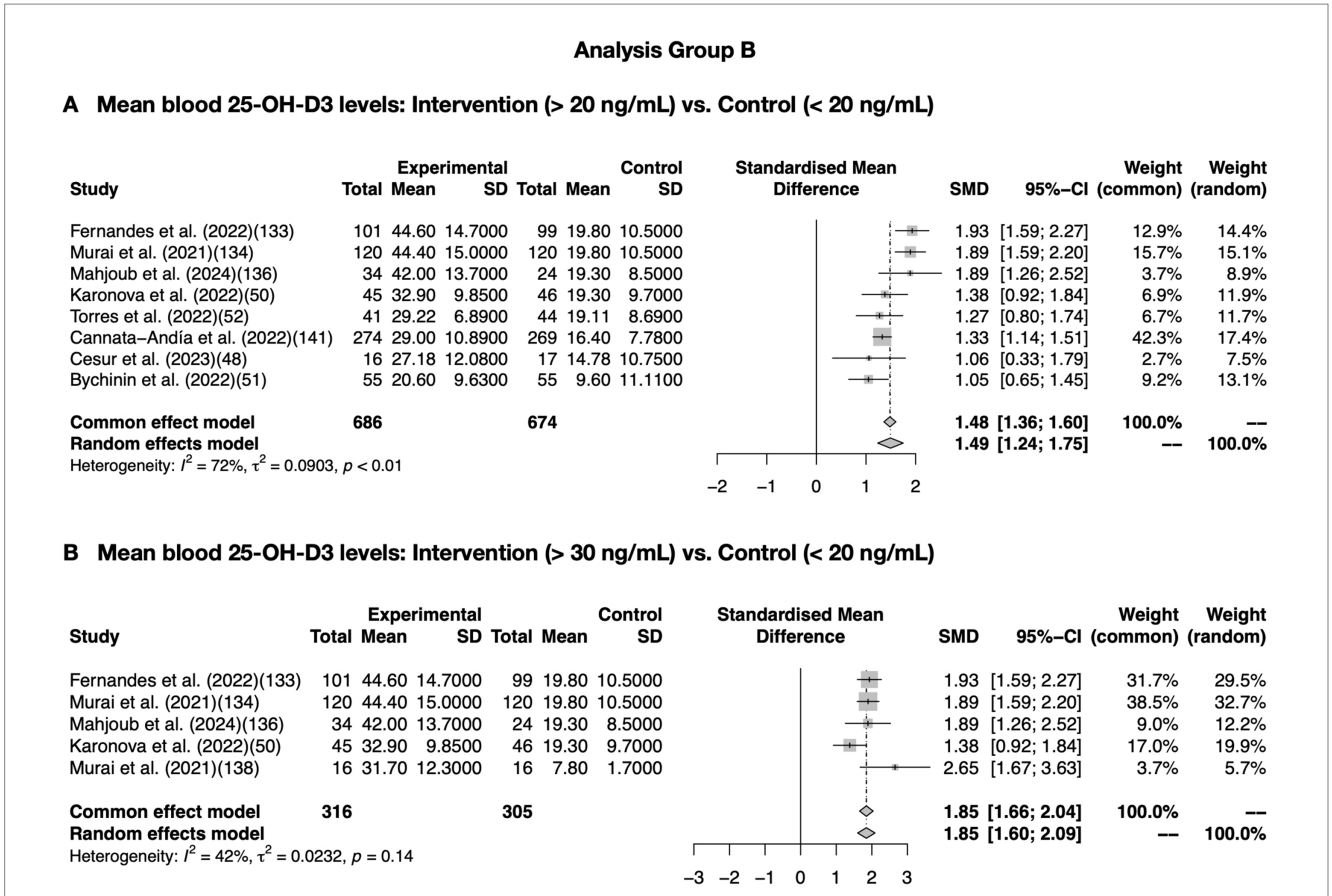

FIGURE 3
Meta-analysis of association between COVID-19 and vitamin D supplementation in Analysis Group *C*. *meta*-analysis conducted using data extracted from articles in Tables 3–5. Meta-analysis was performed only on studies that met both conditions: blood vitamin D levels of 20 (ng/mL) or higher in the intervention group and 20 or lower in the control group. **(A)** Showed a meta-analysis $<I^2$ = 75% of blood 25-OH-D levels extracted from articles in Tables 3–5. **(B)** Further shows meta-analysis in combinations of the data of enough (>30 ng/mL) blood 25-OH-D levels resulted in I^2 = 0%. $p < 0.01$ was considered as a significant difference. $p < 0.01$ were considered as statistical significance.

TABLE 6 Analysis Group C: Effectiveness of a healthy diet against COVID-19 (COVID and polyphenol) RCT.

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
Ahmadi et al. (2023) (35)	Iran	Curcumin	Intervention (n = 29), Control (n = 39), four times/day, 2 weeks	Curcumin with standard COVID-19 treatment enhanced anti-inflammatory effects and reduced the recovery time in mild-to-moderate hospitalized patients	Curcumin improves the time and demand of oxygen therapy and blood Oxygen saturation levels.
Rodríguez-Argente et al. (2023) (36)	Spain	High polyphenolic olive oil	Intervention (n = 44), Control (n = 40) two times/day (2 mL), 3 months	Reduced median recovery time in high polyphonic olive oil intervention, (3 days vs. 7 days)	Daily high polyphenol olive oil significantly reduces the time of recovery.
McCreary et al. (2022) (37)	USA	Resveratrol	Intervention (n = 50), Control (n = 50), 3 weeks	Phase 2 study with resveratrol vs. control: Hospitalization (2 vs. 6%), COVID-19 related ER visits (8 vs. 14%)	Resveratrol is effective in the therapy and other respiratory infectious viruses (influenza, Respiratory Syncytial Virus, and Human Rhinovirus).
de Ligt et al. (2021) (38)	Netherlands	Resveratrol	Crossover trial, Obese male, (n = 11), 30 days	Resveratrol significantly reduces ACE2 (−40%) and leptin (−40%)	Resveratrol reduces ACE2 expression in adipose tissue.

(Figure 4D) contained these events; the total numbers are also illustrated. Statistically significant differences ($p < 0.01$) were observed in blood 25-OH-D3 levels (ng/mL) and number of COVID-19 cases.

3.4.3 Verification of the association between COVID-19 and gut microbiota

Table 9 summarizes eight relevant RCTs out of the 12 searched for COVID and gut microbiota. All the retrieved RCTs focused on the

TABLE 7 Analysis Group C: Effectiveness of a healthy diet against COVID-19 (COVID and flavonoids) RCT.

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
Shohan et al. (2022) (39)	Iran	Quercetin	Intervention ($n = 30$), Control ($n = 30$), 7 days	Quercetin to Remdesivir or Favipiravir treatment significantly reduce hospitalized period, serum ALP, q-CRT, LDH	Quercetin effectively reduced COVID-19 markers (serum ALP, q-CRP, LDH) in severe cases.
Aryan et al. (2022) (40)	Iran	Silymarin	Intervention ($n = 25$), Control ($n = 25$), 3 times/d, 2 weeks	Significant reduction of alanine aminotransferase ($p < 0.001$)	Recommendation of further clinical trials.
Versace et al. (2023) (41)	Italy	Luteolin	Intervention ($n = 17$), Control ($n = 17$), 8 weeks	Palmitoylethanolamide (PEA)-LUT restores GABAB neurotransmission and cortical plasticity.	PEA-LUT recovers cognitive problems in long-COVID associated disorder patients.
Di Stadio et al. (2023) (42)	Italy	Luteolin	Training + Control ($n = 38$), PEA-LUT 1 times/d ($n = 48$), PEA-LUT 2 times/d ($n = 40$), Training + PEA-LUT ($n = 76$), 90 days	PEA-LUT significantly improve olfactory perception in long-COVID patients ($p < 0.0001$)	Olfactory training and PEA-LUT combined recovers over 6 months of olfactory perception disorders in long-OVID patients.
De Luca et al. (2022) (43)	Italy	Luteolin	($n = 69$: Female 43: Male 26), 3 months	Subjects in 37.7% ($n = 26$) had mental clouding but severity decreased after 3 months ($p = 0.02$)	PEA-LUT and olfactory training improve memory function in long-COVID associated and chronic olfactory loss.
Di Stadio et al. (2022) (44)	Italy	Luteolin	Intervention ($n = 130$), Control ($n = 55$), 90 days	Improvement of olfactory disorders in intervention (92 vs. 43%)	Combined PEA-LUT with olfactory training improve more individuals with long-COVID associated olfactory disorders than only olfactory trained individuals.
D'Ascanio et al. (2021) (45)	Italy	Luteolin	Intervention ($n = 7$), Control ($n = 5$), 30 days	Significant improvement in olfactory threshold, discrimination, and identification score ($p = 0.01$)	Combination of PEA-LUT and rehabilitation are associated with the improvement of olfactory functions, especially in significant in patients with long olfactory disorders.
Carrouel et al. (2021) (46)	France	β-cyclodextrin and citrox (bioflavonoids) (CDCM)	Intervention ($n = 88$), Control ($n = 88$), 7 days	Significant decrease of SARS-Cov-2 in saliva after 4 h of first CDCM use ($p = 0.036$), effects continued after 7 days.	Daily use of mouthwash holding CDMC reduces viral load in saliva.

effects of prebiotics and probiotics on COVID-19. Two RCTs from China reported a probiotic, SIM01. According to the literature, the symbiotic formulation of SIM01 contains three bacterial strains: *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, and *Bifidobacterium longum*, and three prebiotic compounds. The first study reported that SIM01 improved intestinal microbiota imbalance (56), and the second reported the alleviation of symptoms in patients with acute post-acute COVID-19 syndrome (PACS) (57). In another report, an aqueous extract of *Dendrobium officinale* (DoAE) was found to reduce inflammatory gut microbiota (58). Additionally, a Mexican study reported that probiotics containing *Lactiplantibacillus plantarum* and *Pediococcus acidilactici* enhance antibody production against COVID-19 by interacting with the host immune system (59). Similarly, a study conducted in the UK found that probiotics, including *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, and *Bifidobacterium animalis* subsp. *lactis*, reduced the symptoms of viral upper respiratory tract infections (URTI) symptoms by 27% in overweight/obese subjects (60). In Sweden, probiotics containing *Limosilactobacillus reuteri* have been reported to increase antibody production following vaccination compared to vitamin D alone (61). In a Spanish study, probiotics and prebiotics improved the cardiometabolic profile (62), and in the USA, prebiotic fibers were shown to affect the gut microbiota associated with serum serotonin production and help improve mental health during long COVID (63).

Thus, the gut microbiota plays a significant role in improving COVID-19 outcomes and sequelae, as evidenced by a systematic review.

3.5 Analysis group D: Association between healthy diet, phytochemicals, vitamin D, and gut microbiota

3.5.1 Association of polyphenols or flavonoids with gut microbiota

The results of the PubMed search for polyphenols, flavonoids, and the gut microbiota are shown in Table 10. As there were 30 RCTs

TABLE 8 Analysis Group C: Association between blood vitamin D levels and COVID-19 mortality (COVID and vitamin D) RCT.

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
Annweiler et al. (2022) (47)	France	Single oral high dose vitamin D3 (400,000 IU) or Standard dose (50,000 IU), after COVID-19 diagnosis in 72 h	400, 000 IU ($n = 127$), 50,000 IU ($n = 127$)	Clear benefit in 14 days COVID-19 death (6% vs. 11%)	Early vitamin D3 (400, 000 IU) supply reduced deaths in elderly patients.
Cesur et al. (2023) (48)	Turkey	Single oral vitamin D3 (150,000 IU) or Control, after COVID vaccination	150,000 IU ($n = 16$: 14 Pfizer-BioNTech, 2 Sinovac), Control ($n = 17$: 14 Pfizer-BioNTech, 3 Sinovac)	Significant increase of serum IgG, difference between IgG and serum 25-OH-D3in supplementation period	Vitamin D3 (150,000 IU) upregulate immune response and effective in vaccine-induced antibody levels.
Sarhan et al. (2022) (49)	Egypt	Intramuscular high dose vitamin D3 (200,000 IU/d) or Oral low dose alphacalcidol (active form of vitamin D3) (40 IU/d), at least consecutive 5 days	200,000 IU/d vitD3 ($n = 58$), 40 IU/d alphacalcidol ($n = 58$)	Significantly shortened hospitalization (8.6 vs. 6.8d), reduced necessity of high-oxygen and non-invasive mechanical ventilator (67 vs. 33%), clinical improvement (45 vs. 55%), onset of sepsis (64 vs. 33%)	Vitamin D3 (200,000 IU/d) is effective in cytokine storms and fewer adverse outcomes.
Karonova et al. (2022) (50)	Russia, USA	Oral high dose vitamin D3 (50,000 IU/w), 2 weeks and (5,000 IU/d), 3 months, or Standard dose (2,000 IU/d), 3 months	50,000 IU/w + 5,000 IU/d ($n = 45$), 2,000 IU/d ($n = 46$)	Only 26% in high dose onset asymptomatic COVID-19 but twice in standard dose.	Vitamin D3 (50,000 IU/w + 5,000 IU/d) is effective and safe to achieve enough blood 25-OH-D3 level.
Karonova et al. (2022) (143)	Russia	Oral vitamin D3 (50,000 IU/d) or Control, clinical features and inflammation markers in COVID-19 patients, 1 and 8 days of hospitalization	50,000 IU/d ($n = 56$), Control ($n = 54$)	Significant difference in serum 25-OH-D3 levels ($p < 0.001$), high neutrophil and lymphocyte counts ($p = 0.04$; $p = 0.02$), low CRP level ($p = 0.02$)	Vitamin D3 (50,000 IU) increases serum 25-OH-D3 levels with positive effects.
Bychinin et al. (2022) (51)	Russia	Oral vitamin D3 (60,000 IU/w) and (5,000 IU/d), or Control, 7 weeks	60,000 IU/w + 5,000 IU/d ($n = 55$), Control ($n = 55$)	Significantly higher NK and NKT cell counts and neutrophil-to-lymphocyte ratio (NLR) on day 7	Vitamin D3 (60,000 IU/w + 5,000 IU/d) significantly increased lymphocyte numbers.
De Niet et al. (2022) (144)	Belgium	Oral vitamin D3 or Control, (25,000 IU/d), 4 days and (25,000 IU/w), maximum 6 days	25,000 IU ($n = 50$), Control ($n = 50$)	Low hospitalized rate after 7 days (19 vs. 54%; $p = 0.0161$), hospitalized patients' numbers at day 21 (0 vs. 14), reduced oxygen supply (4 days vs. 7), significant reduction of WHO scale	Vitamin D3 (25,000 IU) improved clinical outcome in hospitalized patients.
Torres et al. (2022) (52)	Spain	Oral vitamin D3 high dose (10,000 IU/d) or Moderate dose (2,000 IU/d), 2 weeks	10,000 IU/d ($n = 41$), 2,000 IU/d ($n = 44$)	Increase of average serum 25-OH-D3 levels (29 vs.19 ng/mL; $p < 0.0001$)	Addition of vitamin D3 (10,000 IU/d) to the standard treatment shorten the period of hospitalization and improve the prognosis.
Sabico et al. (2021) (53)	Saudi Arabia	Oral vitamin D3 high dose (5,000 IU/d) or Standard dose (1,000 IU/d), middle to moderate COVID-19 patients, 2 weeks	5,000 IU/d ($n = 36$), 1,000 IU/d ($n = 33$)	Significantly increases serum 25-OH-D3 levels ($p = 0.003$)	2 weeks of daily oral vitamin D3 (5,000 IU/d) shorten the recovery time of coughing and taste loss.
van Helmond et al. (2022) (145)	USA	Oral vitamin D3 (5,000 IU/d) or Control, in healthcare workers with influenza-like illness (ILI), at least 2 months	5,000 IU/d ($n = 255$: 47 ± 12 years old, Female 99), Control ($n = 2,827$)	Significantly reduces ILI risks and non-COVID ILI incidence	Vitamin D3 (5,000 IU/d) alleviates influenza-like illness in healthcare workers.

(Continued)

TABLE 8 (Continued)

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
LaRiccia et al. (2023) (54)	USA	Oral vitamin D3 (5,000 IU/d) or Control, in 9 months	5,000 IU/d ($n = 196$), Control ($n = 1958$)	Reduced healthcare utilization due to COVID-19 (rate difference: -8.47×10^{-3} per 1,000 person-days)	Vitamin D3 (5,000 IU/d) reduced hospitalizations due to COVID-19.
Villasis-Keever et al. (2022) (142)	Mexico	Oral vitamin D3 (4,000 IU/d) or Control, 30 days follow up	4,000 IU/d ($n = 94$), Control ($n = 98$)	Reduction of SARS-CoV-2 infection (6.4 vs. 24.5%; $p < 0.001$), lowered inflation risks, kept high serum 25-OH-D3 levels irreverent to vitD3 deficiency.	Vitamin D3 (4,000 IU) prevents SARS-CoV-2 infection.
Caballero-García et al. (2021) (139)	Spain	Oral vitamin D3 (2,000 IU /d) or Control, 6 weeks	2,000 IU/d ($n = 15$), Control ($n = 15$), Male	Optimized serum creatine kinase levels and protective effects for muscle catabolism	Vitamin D3 (2,000 IU) reduces the muscle damage indicators and improve the health status and QOL in recovery period.
Elamir et al. (2022) (146)	Israel	Oral calcitriol (active form of vitamin D3) (20 IU/d) or Control, 2 weeks	20 IU/d calcitriol ($n = 50$), Control ($n = 50$)	Increase of peripheral arterial oxygen saturation to the inspired fraction of oxygen (SaO_2/FIO_2 ratio) in intervention (+91.04 vs. +13.21)	Calcitriol (20 IU/d) intervention improves blood oxygen saturation in hospitalized patients.
Dilokpattanamongkol et al. (2024) (147)	Thailand	Oral alfacalcidol (active form of vitamin D3) (80 IU/d) or Control, COVID-19 patients, until discharge	80 IU/d alphacalcidol ($n = 147$), Control ($n = 147$)	Significant reduction of pneumonia severity index ($p = 0.007$) and CRP in patients over 30 mg/L ($p < 0.001$)	Addition of active vitamin D3 (80 IU/d) to the standard treatment is beneficial to the patients requiring oxygen supplementation, high dose corticosteroid therapy or patients with high CPR (> 30 mg/L).
Entrenas Castillo et al. (2020) (55)	Spain	Oral 25-OH-D3 or Control, day1 (20,000 IU), day 3 and 7 (10,000 IU), COVID hospitalized patients	20,000 IU 25-OH-D3 ($n = 50$), Control ($n = 26$)	Intervention: none died, all discharged without complications. Control: all not admitted to the ICU discharged. Of the 13 patients admitted to the ICU, two died and remaining 11 discharged.	25-OH-D3 (20,000 IU) intervention reduces the severity.
Bishop et al. (2023) (132)	USA	Oral extended-release 25-OH-D3 or Control, in COVID-19 patients, (12,000 IU/d) day 1–3 and (2,400 IU/d) day 4–27	12,000 IU 25-OH-D3 ($n = 65$), Control ($n = 69$)	Serum 25-OH-D3 > 50 ng/mL (81 vs. 15%; $p < 0.0001$)	Serum 25-OH-D3 levels became >50 ng/mL in outpatients, improve the prognosis and reduce the risk of pneumonia.
Maghbooli et al. (2021) (148)	Iran	Oral 25-OH-D3 (around 3,000–6,000 IU/d) or Control, hospitalized COVID-19 patients of blood 25-OH-D3 lower than 30 ng/mL	25-OH-D3: Control, Assigned (53:53), First month (34:24), 2nd month (24:19)	Increased lymphocyte populations and reduces neutrophil/lymphocyte ratio, low neutrophil/lymphocyte ratio is associated with ICU admission days and mortality.	Oral 25-OH-D3 upregulate immune responses through lymphocyte population and correct vitamin D deficiency in patients.
Mahjoub et al. (2024) (136)	Tunisia	Supplement (zinc, multivitamin and melatonin) or Control, treatment of COVID-19 and similar symptoms in 30 days	Intervention ($n = 88$), Control ($n = 87$)	Complete recovery (80.5 vs. 67.1%; $p = 0.038$)	Melatonin, zinc, and vitamins shorten the recovery time in and other diseases.
Reino-Gelardo et al. (2023) (149)	Spain	Food supplement (probiotics, prebiotics, vitamin D, zinc, and selenium), in hospitalized COVID-19 patients or control	Intervention ($n = 70$), Control ($n = 69$)	Shorter digestive symptoms (2.6 vs. 4.3 days; $p = 0.001$), shorter hospital stay of non-severe disease on chest X-ray patients (8.1 vs. 11.6 days; $p = 0.007$).	Food supplement (Gasteel Plus®) was protective factor and shorten the recovery of GI symptoms.

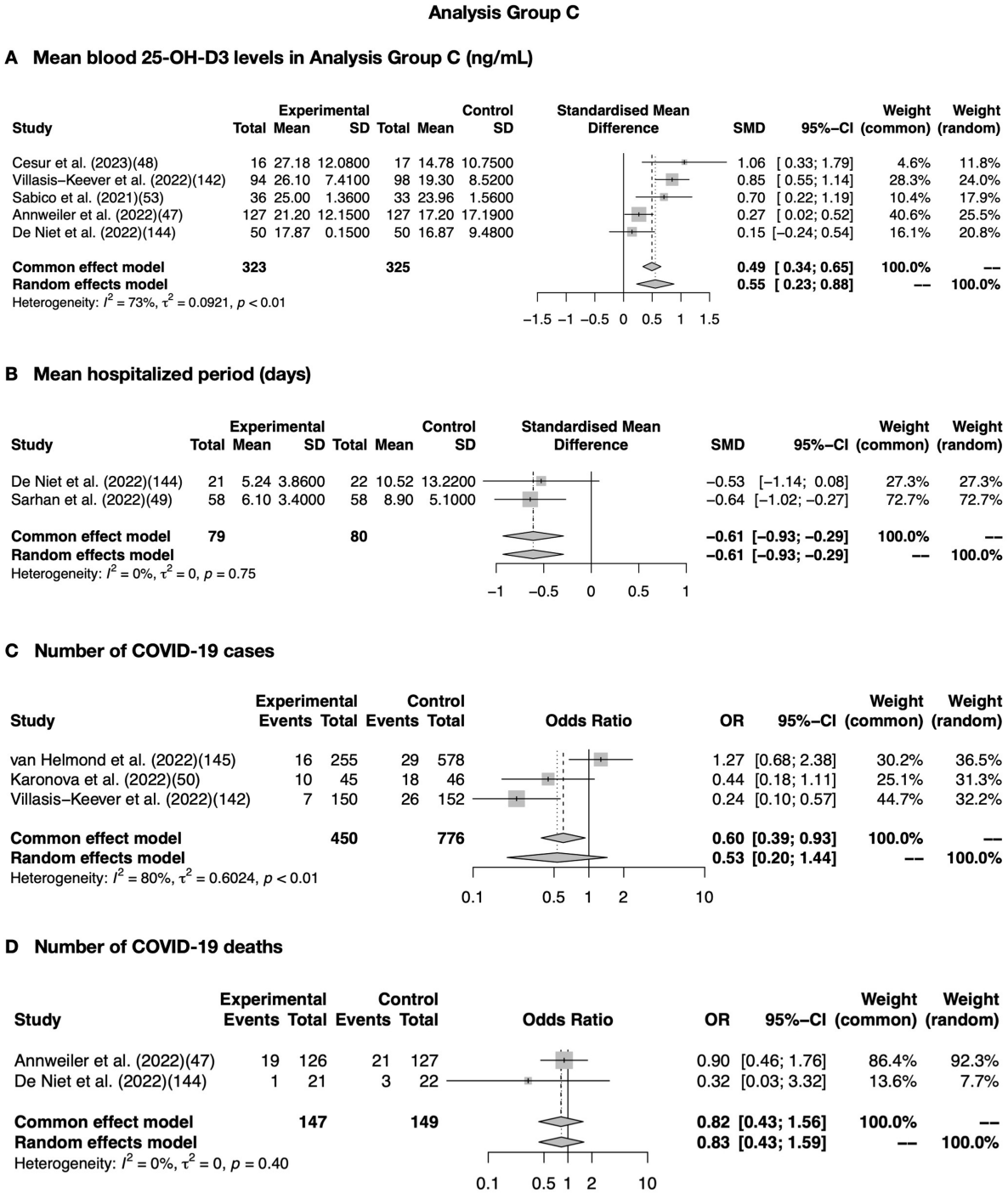

FIGURE 4
Meta-analysis of association between COVID-19 and vitamin D supplementation in Analysis Group B. Meta-analysis conducted using data extracted from articles in Table 8. **(A)** Show a meta-analysis of serum 25-OH-D levels combination of $<I^2 = 75\%$. **(B)** Shows a meta-analysis of the difference of mean hospitalized period extracted from articles shown in Table 8. **(C)** Shows a meta-analysis of COVID cases; data was shown in odds ratio. **(D)** Shows a meta-analysis of COVID deaths. $p < 0.01$ was considered as a significant difference.

reporting these associations over a 5-year period, the seven main articles from the past year are listed below.

A study from New Zealand indicated a relationship between the intake of rutin-supplemented yogurt and an increase in the number of butyrate-producing bacteria and decrease in fasting blood glucose levels (64). In an American study, xanthohumol found in hops reduced bile acid metabolism via microbiota specific to the gut forms of *Prevotella* and *Ruminococcus* (65). Functional foods containing anthocyanins increased *Bifidobacterium* and improved cognitive function and eye

TABLE 9 Analysis Group C: Association between COVID-19 and gut microbiota (COVID and gut microbiota) RCT.

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
Wong et al. (2023) (56)	China	Probiotics (SIM01) after initial COVID-19 vaccination within a week, 3 months	Intervention ($n = 224$), Control ($n = 229$)	SIM01 improves quality of sleep ($n = 53$ vs. 22), improvement in skin condition ($n = 18$ vs. 8), better mood ($n = 27$ vs. 13)	Probiotics SIM01 recover dysbiosis in diabetic patients and elderly in pandemic.
Lau et al. (2024) (57)	China	Probiotics (SIM01: 10 billion CFU/d), in post-acute COVID-19 syndrome (PACS) patients, 1 time/d, 6 months	Intervention ($n = 232$), Control ($n = 231$)	Recovery from fatigue (OR 2·273, 95% CI 1·520–3·397, $p = 0·0001$), memory loss (1·967, 1·271–3·044, $p = 0·0024$), difficulty in concentration (2·644, 1·687–4·143, $p < 0·0001$), gastrointestinal upset (1·995, 1·304–3·051, $p = 0·0014$), general unwellness (2·360, 1·428–3·900, $p = 0·0008$)	Probiotics SIM01 reduced several PACS symptoms.
Gao et al. (2023) (58)	China	Upregulation of immune response with *Dendrobium officinale* aquatic extract (DoAE) supplementation, in healthy subjects after COVID vaccination, 9 weeks	Intervention ($n = 39$), Control ($n = 30$)	Significant increase of physical performance, sleep, mental performance, appetite, IFN-γ production, and the number of *Faecalibacterium*	DoAE upregulate immune responses, decrease inflammatory gut microbiota and dysbiosis.
Gutiérrez-Castrellón et al. (2022) (59)	Mexico	Probiotics (*Lactiplantibacillus plantarum* KABP022, KABP023, KAPB033 strain, *Pediococcus acidilactici* KABP021 strain: total 2×10^9 CFU), 30 days	Intervention ($n = 147$), Control ($n = 146$)	Significantly increased SARS-Cov-2 specific IgM and IgG	Probiotics not only changed the gut microbiota in colon but also interact with host immune system.
Mullish et al. (2021) (60)	UK	Influence of probiotics in viral upper respiratory tract infections (URTI), 6 months	BMI 25–34.9 kg/m^2, 30–65 years old ($n = 220$)	Significantly reduced URTI symptoms by 27%, especially in subjects over 45 years old and BMI 30 kg/m^2	Probiotics prevents viral URTI especially in overweight/obese people.
Forsgård et al. (2023) (61)	Sweden	Probiotics (*Limosilactobacillus reuteri* DSM 17938: smallest 1×10^8 CFU) + vitD3 (10 µg /d), control was supplied only vitD3, 2 times/d, 6 months	Participants ($n = 159$), Completion of 3 times of research visit ($n = 132$)	In intention-to-treat (ITT) analysis, COVID positive individuals ($n = 6$) have higher serum anti-spike IgG (6,09 L vs. 111 BAU/mL) and anti-receptor binding domain IgG (928 vs. 83.7 BAU/mL)	Probiotics strengthen IgA response in mRNA based COVID vaccinated patients.
Sevillano-Jiménez et al. (2022) (62)	Spain	Nutritional education program with high symbiotic foods (dairy products, fermented foods, green-yellow vegetables, high-fiber, and whole grains), 6 months	Intervention ($n = 23$), Control ($n = 21$)	Statistical differences in all anthropometric variables, 27.4% reduction in the prevalence of metabolic syndrome risk factors, decrease in cardiovascular risk at 6 months	Probiotics improves cardio metabolic profiles in hospitalized COVID-19 patients with schizophrenia spectrum disorders.
Blackett et al. (2022) (63)	USA	Prebiotics-fibers in GI symptoms and mental health symptoms after COVID-19, 6 months	(i) Faecal samples from patients with acute COVID-19, (ii) blood samples from patients with acute COVID-19	Blood serotonin synthesis associated reduced biosynthesis of L-tryptophan by the gut microbiota affects severe GI symptoms	Reduction of serotonin signaling associated gut microbiome is associated with persistent GI symptoms and mental health in long-COVID.

dryness (66). Additionally, autologous fecal transplantation was effective in weight loss among consumers of a high-polyphenol Green Mediterranean Diet (67). An interesting finding was the inverse association between the Green Mediterranean diet and biological aging in the group with increased polyphenol intake (68).

These results highlight the significant impact of polyphenols and flavonoids on the gut microbiota composition and related health outcomes. Modulation of the gut microbiota by these compounds may offer protective benefits and improve overall health, particularly in the context of dietary interventions aimed at enhancing gut health and preventing disease.

TABLE 10 Analysis group D: Association of polyphenols or flavonoids with gut microbiota (polyphenol or flavonoids and gut microbiota) RCT last 1 year.

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
Mathrani et al. (2023) (64)	New Zealand	Rutin	Rutin supplemented yogurt 500 mg/d ($n = 24$), Rutin capsule ($n = 25$), Control ($n = 24$), 12 weeks	Fasting blood glucose has inverse relationship with butyrate-producing *Roseburia inulinivorans* abundance	First examination of after meal pancreatic β-cell function with rutin.
Jamieson et al. (2024) (65)	USA	Xanthohumol (XN)	NX 24 mg/d ($n = 16$), Control ($n = 14$), 8 weeks	Re-shape of individual taxa in an enterotype-dependent manner	Reductions in microbiota-derived bile acid metabolism specific to *Prevotella* and *Ruminococcus* enterotypes were derived.
Tosi et al. (2023) (150)	Italy, UK	Cranberry (poly) phenol	Freeze dried cranberry powder ($n = 31$), Control ($n = 29$), 12 weeks	Cranberry was associated with the changes of blood polyphenol metabolites levels	Cranberry polyphenol is associated with the health improving effects.
Lackner et al. (2024) (151)	Austria	Aronia	Natural aronia juice ($n = 20$), Control ($n = 20$), Female, twice/day, 6 weeks	Intervention group was divided into tolerant (Vt) and intolerant (Vc), Vt significantly changed microbiome diversity	Aronia juice polyphenol had personally different responses for gut microbiota.
Wattanathorn et al. (2023) (66)	Thailand	Anthocyanin	Intervention (4 g) ($n = 23$), (2 g) ($n = 23$), Control ($n = 23$), 8 weeks	Cognitive function↑, Working memory↑, Eye dryness↓, *Bifidobacterium* spp.↑	Anthocyanin holding supplement (Anthaplex) increased *Bifidobacterium* spp. and improved cognitive function and symptom of dry eyes
Kamer et al. (2023) (67)	Israel, UK, USA, France, Germany	High-polyphenol green Mediterranean diet	aFMT ($n = 41$), Control ($n = 41$), 6 months	High gut microbiota diversity participants avoid recovery of body weight increase for 8–14 months (−0.58 ± 2.4 vs. 3.18 ± 3.5 kg; $p = 0.02$)	High-polyphenol green Mediterranean diet was effective in the decrease of bodyweight in autologous-fecal-microbiota-transplantation (aFMT).
Yaskolka Meir et al. (2023) (68)	Israel, USA, France, Germany	Polyphenol rich low red/ processed meet green Mediterranean diet (MED)	Green-MED ($n = 87$), MED ($n = 81$), Control ($n = 88$), Green-MED include green tea (3–4 cup/d) with Wolffia green shake (500 mL) (+800 mg/d polyphenol), Both MED groups take walnuts (28 g/d) (+440 mg/d polyphenol)	MED intervention improves DNA methylation age (mAge) - 8.9 months ($p = 0.02$)	High-polyphenol intake in MED had inverse association between biological ageing.

3.5.2 Association between vitamin D and gut microbiota

The results of the PubMed search for vitamin D and the gut microbiota are shown in Table 11. Several recent RCTs conducted over the past 5 years have demonstrated that vitamin D supplementation significantly affects changes in the gut microbiota. One year of supplementation with vitamin D (2,000 IU/day) in patients with colorectal cancer (CRC) resulted in a significant increase in *Leuconostoc pseudomesenteroides*, *Ruminococcus* YE78, *Faecalibacterium prausnitzii*, and *Bacteroides clarus* (69). Additionally, 16 weeks of vitamin D3 supplementation in vitamin D-deficient, overweight/obese individuals led to an increase in *Lachnospira* spp., a decrease in *Blautia* spp., and an increase in *Coprococcus* spp., while decreasing *Ruminococcus* spp. in groups with high serum vitamin D levels (70). Intramuscular vitamin D3 (200,000 IU) increased *Bifidobacteriaceae* and *Christensenellaceae* and decreased *Proteobacteria* after 8 weeks (71). Other findings suggest that increased vitamin D levels during pregnancy protect against the growth of sulfate-reducing bacteria such as *Desulfovibrio*, which are associated with chronic intestinal inflammatory disorders (72). Studies on vitamin supplementation, including that of vitamin D, have also shown increased microbial alpha diversity and short-chain fatty acids (73).

These findings highlight the significant role of vitamin D in modulating the gut microbiota, which may have implications for overall health and management of diseases related to gut health. The beneficial effects of vitamin D on the gut microbiota composition suggest its potential therapeutic application, particularly in conditions involving gut dysbiosis and inflammatory disorders.

3.5.3 Relevance of phytochemicals and vitamin D

The association between vitamin D, an essential nutrient, and polyphenols and flavonoids, the main components of the

TABLE 11 Analysis group D: Association between vitamin D and gut microbiota (vitamin D and gut microbiota) RCT last 5 years.

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
Bellerba et al. (2022) (69)	Italy	Vitamin D (2,000 IU/d), 1 year	Intervention ($n = 32$), Control ($n = 28$)	*Leuconostoc pseudomesenteroides*↑, *Ruminococcus* YE78↑, *Faecalibacterium prausnitzii*↑, *Bacteroides clarus*↑	Vitamin D participates in gut microbiota formation and gut microbiota is associated with the efficacy of 25-OD-D3 in colorectal cancer (CRC) patients.
Naderpoor et al. (2019) (70)	Australia	Vitamin D3(100,000 IU), and (4,000 IU/d), every day, 16 weeks	Intervention ($n = 14$), Control ($n = 12$), Vitamin D deficiency, Overweight/obese	Genus *Lachnospira*↑, genus *Blautia*↓, In high 25-OD-D3 subjects, genus *Coprococcus*↑, genus *Ruminococcus*↓	Vitamin D3 significantly affects in several fecal gut microbiota.
Lee et al. (2022) (71)	South Korea	Intramuscular vitamin D3 (200,000 IU)	Intervention ($n = 8$), Control ($n = 10$)	In recovery, Microbial alfa diversity↑, *Proteobacteria*↓, *Lachnospiraceae*↑, *Ruminococcaceae*↑, *Akkermansiaceae*↑, *Bifidobacteriaceae*↑ After 8 weeks, *Bifidobacteriaceae*↑, *Christensenellaceae* ↑, *Proteobacteria* ↓	High dose intramuscular vitamin D3 influences gut microbiota in patients with *Clostridioides difficile* infection.
Aparicio et al. (2023) (72)	USA	Vitamin D3 (4,400 IU/d) for pregnant women	($n = 114$)	Maternal gut microbiome is not changed by vitamin D and pregnant women have high genus *Desulfovibrio* population.	Increased vitamin D level during pregnancy could be protective against the growth of sulfur-reducing bacteria such as *Desulfovibrio*.
Pham et al. (2021) (73)	Switzerland	Vitamin A, B2, C, D, E	Vitamin A, B2, C, B2 + C, D3, E ($n = 12$) each, Control ($n = 24$)	Microbial alfa diversity↑, Fecal short fatty acid↑, Vitamin C had the largest effect	Follow-up studies with vitamins to the colon may help clarify the clinical significance of gut microbiota.

phytochemical group, shown in Table 12 after a PubMed search. Several studies have highlighted synergistic effects of these nutrients.

First, curcumin supplementation led to significant improvements in blood vitamin D levels and liver function enzyme levels in women with premenstrual syndrome (PMS) and dysmenorrhea (74). Second, the osteoprotective effect of resveratrol was greater in the participants who were supplemented with vitamin D and calcium (75). Additionally, supplementation of the Mediterranean diet with apple and bergamot juices in an Italian study reduced the risk of chronic noncommunicable diseases (CNCD) and increased VDR gene expression (76). Silymarin combined with vitamin D improves nonalcoholic fatty liver disease (NAFLD) (77). Furthermore, a combination of alpha-lipoic acid, acetyl-L-carnitine, resveratrol, and vitamin D3 supplementation with rehabilitation was effective for sciatica (78). *Perilla frutescens* dried seed extract, containing quercetin and vitamin D3, has also been shown to be effective against pediatric allergic rhinitis (79).

As described above, a link was observed between dietary factors, phytochemicals, vitamin D, and the gut microbiota, as well as between phytochemicals and vitamin D. These findings indicated that a healthy dietary pattern is an important long-term protective factor against pneumonia, including COVID-19.

4 Discussion

This systematic review first examined whether a healthy diet was effective against COVID-19. Japan has the longest longevity in the world, and in recent years, there has been growing awareness of the need to reduce medical costs and prevent aging, particularly by detecting and curing non-disease conditions (ME-BYO) (80). Consequently, considerable research has been conducted on healthy longevity and diet (81). Unlike the typical high-fat, high-sugar Western diet, the Japanese diet, which is similar to the Mediterranean diet, is well-known worldwide as a healthy diet, making those who consume it less prone to metabolic-related diseases and obesity (82).

In developed countries, high infection and mortality rates owing to COVID-19 have been observed, particularly among the elderly and those with underlying diseases, who constitute a large proportion of the population. Consequently, these groups are prioritized for vaccination (83). A healthy Japanese diet may be associated with lower rates of COVID-19 due to the lower prevalence of underlying lifestyle-related diseases in the population.

Africa, on the other hand, is a region with a poor food situation, including hunger and water shortages, and the economic impact of COVID lockdowns and other problems was a major

TABLE 12 Analysis group D: Relevance of phytochemicals and vitamin D (polyphenol or flavonoids and vitamin D) RCT last 5 years.

First author, year, references	Country	Treatment	Subjects	Main findings	Outcome
Arabnezhad et al. (2022) (74)	Iran	Curcumin	Intervention (n = 38), Control (n = 38), (Curcuminoid 500 mg + piperine 5 mg), every day, from approximately 7 days before until 3 days after menstruation for three consecutive menstrual cycles	Blood 25-OH-D3↑, Aspartate aminotransferase↓, Bilirubin↓	Curcumin improved serum 25-OH-D3 levels and liver function enzyme test results in premenstrual syndrome (PMS) and dysmenorrhea women
Wong et al. (2020) (75)	Australia	Resveratrol and vitamin D3	Intervention (n = 73), Control (n = 73), twice/day, 24 months, crossover trial	Bone density in lumbar spine and neck of femur↑, Bone absorption marker: C-terminal telopeptide type-1 collagen levels↓	Bone protective effects of resveratrol were larger in subjects taking vitamin D and calcium.
Gualtieri et al. (2019) (76)	Italy	Mixed apple and bergamot (MAB) juice addition to Mediterranean diet	(n = 24: in 16 Female), 2 weeks	Gain in lean mass↑, Total cholesterol/HDL index↓, MIF↑, PPARγ↑, SOD1↑, VDR↑	MAB juice addition to Mediterranean diet reduced the risk of chronic non-communicative diseases (CNCDs), and increased VDR gene expression.
Federico et al. (2019) (77)	Italy	Silymarin and vitamin D	Intervention (n = 60), Control (n = 30), 6 months	Metabolic markers↓, Endothelial dysfunction↓, Oxidative stress parameters↓, worsening of disease↓, after 6 months	Silymarin and vitamin D containing supplements (RealSIL 100D®) improves NAFLD.
Scaturro et al. (2023) (78)	Italy	Resveratrol and vitamin D3	Combo: Rehabilitation + Supplementation (Alpha Lipoic Acid、600 mg, Acetyl-L-Carnitine 1,000 mg, Resveratrol 50 mg, Vitamin D3 800 IU), Rehabilitation alone, Supplement alone	Pain↓, QOL↑, in combo group	Combined administration of resveratrol and vitamin D3 with rehabilitation are effective in sciatica.
Marseglia et al. (2019) (79)	Italy	Quercetin, vitamin D3, *Perilla frutescens* dried seed extract holding food supplement	Intervention (n = 64), Control (n = 64), Children, 4–12 weeks of Phase II	Halved allergic rhino conjunctivitis (AR) risks (HR = 0.54)	Quercetin, vitamin D3, *Perilla frutescens* dried seed extract containing food supplement (Lertal®) improves childhood AR.

concern (84). However, contrary to our expectations, the reported number of deaths due to COVID-19 did not increase significantly. One reason is that since Africa's situation cannot be compared with that of other regions owing to underdeveloped health systems, the COVID response in Africa has been underreported. In addition, regional differences exist between the urban and non-urban areas in Africa. However, the proportion of younger people is the highest in the world, and the proportion of patients with metabolic syndrome, lifestyle-related diseases, and diseases caused by overeating is lower than in developed and emerging countries (85). This is associated with the fact that COVID-19 mortality rates were low in Africa.

Vegetables and fruits have received attention in recent years because of their high phytochemical content (86). They are referred to as the seventh nutrient, following the three macronutrients—carbohydrates, proteins, and fats—the fourth and fifth nutrients—vitamins and minerals, and the sixth nutrient–dietary fiber. Phytochemicals have gained particular attention owing to their anti-inflammatory effects and well-known antioxidant properties. Numerous studies have investigated the antibacterial, anti-viral, and anti-cancer properties of these compounds. Grape phytochemicals such as resveratrol are among the most widely studied and used compounds (87). Many other plant-derived ingredients are extensively utilized not only in foods, but also in Kampo (traditional Japanese) and other herbal medications, from aspirin to the antimalarial drug artemisinin.

Next, we examined whether blood vitamin D levels were associated with COVID-19 mortality. Recently, there have been an increasing number of reports on the immune-boosting properties of vitamin D (88). Vitamin D was named after Elmer McCollum in 1922

as the fourth vitamin, with Vindaus et al. contributing to early research. It is well-known for its role as a bone hormone and its involvement in calcium absorption in the intestinal tract. Vitamin D deficiency is well known to be associated with rickets in children (89) and osteoporosis and osteomalacia in the elderly (90).

Vitamin D toxicity can lead to hypercalcemia and calcium accumulation in blood vessels caused by excessive vitamin D intake combined with calcium intake. This effect could be reversed by preventing excessive vitamin D intake. There are two types of vitamin D: plant-derived vitamin D2, produced in mushrooms, and animal-derived vitamin D3. Vitamin D3 is produced in the body from cholesterol precursors in the skin, but the activated form, 1α,25-(OH)2-D3, has a short half-life of a few hours and is not excessive in its natural state.

It is also well known that African Americans living in temperate regions are often deficient in vitamin D3, as its production in skin cells is inhibited by high melanin levels (91). For similar reasons, vitamin D supplementation is recommended, particularly in the UK and Scandinavian countries because of the high prevalence of vitamin D deficiency at higher latitudes (92). As mentioned previously, vitamin D deficiency is much less common in mainland Africa than in other countries. A study comparing East Africa and Finland found that East Africans had a higher vitamin D intake (93), with differences in diet and sunlight exposure across regions being associated (94).

It has also been suggested that in Africa, unlike in developed countries where the population is concentrated in urban areas, there are far more opportunities for exposure to direct sunlight owing to differences in living conditions. Therefore, sufficient vitamin D is synthesized despite the high melanin pigmentation in the skin (95). This phenomenon is attributed to the fact that people living at higher latitudes lose the need for pigments that protect their bodies from direct sunlight.

In recent years, it has been noted that vitamin D deficiency is associated with compromised immunity, since vitamin D receptors (VDRs) expressed in many cells, including immune cells (96). Active vitamin D, bound to the nuclear VDR, binds to the vitamin D response elements of genes and regulates their expression of various genes. This action is particularly prominent in proinflammatory cytokine genes such as TNF-α and IL-1β, thereby providing vitamin D with anti-inflammatory properties and making it deeply involved in immune regulation (97).

There were remarkable numbers of meta-analysis in PubMed search (65 results) associated with the keywords COVID-19 and vitamin D (98). Therefore, a meta-analysis was conducted from the references in Tables 3–5, 8, and the statistical analysis of blood 25-OH-D3 levels, hospitalization period, COVID-19 cases, and deaths in relationship to COVID-19 and vitamin D. Statistically significant differences ($p < 0.01$) in blood 25-OH-D3 levels and number of COVID-19 cases were observed in this study, similar to other meta-analyses. However, it is likely that vitamin D works as supplementary regimen for daily upregulation of immune responses to avoid infections rather than the treatment of severe COVID-19.

Contrary to prior predictions, there was no significant increase in the number of deaths from COVID-19 in Africa despite the high prevalence of other infectious diseases such as AIDS and malaria (99). Although vitamin D deficiency is common in Africa, it is less prevalent than that in other regions of the world. Therefore, it is highly likely that higher average blood vitamin D levels in Africa are associated with improved survival rates. In contrast, mortality from COVID-19 was associated with blood vitamin D levels, similar to trends observed in other regions.

Finally, we examined whether COVID-19 is associated with the gut microbiota. In recent years, gut microbiota have been found to be associated with various diseases (100). The gut microbiota of the Japanese people can be categorized into five types. The gut microbiota phenotype of healthy Japanese individuals is referred to as the rural type and is characterized by high levels of *Prevotella*, which is associated with a reduced risk of various diseases (101). A study of African children found that, compared to their European counterparts, children in rural African villages had an enrichment of *Bacteroides* and a reduction of *Firmicutes*, resulting in a more diverse and healthier gut microbiota (102). This was attributed to the primitive, fiber-rich diet of Africans with healthy low *Firmicutes*/*Bacteroides* (F/B) ratio and linked to the low COVID-19 infection rates and deaths in Africa, presenting a remarkably interesting finding.

Patients with COVID-19 show reduced diversity of microbiota in the lungs, including a reduction in *Bacteroides* (103). Focusing on the gut microbiota, it was found that short-chain fatty acid-producing bacteria, mainly from the class *Clostridia* decreased, whereas opportunistic pathogens increased, resulting in leaky gut syndrome (104). Short-chain fatty acids, such as acetic acid, propionic acid, and butyric acid, are crucial for the activation of regulatory T cells (Tregs) and upregulate immunity. Furthermore, an increase in opportunistic pathogens, including mycoplasmas, has been observed in the respiratory tracts of COVID-19 patients (105). Opportunistic pathogens are normally present in the body but become pathogenic when the immune system is weakened. Thus, a link between COVID-19 and the gut microbiota has been suggested.

In addition, vitamin D helps maintain healthy gut microbiota (106). The composition of the gut microbiota varies greatly depending on the diet and can be broadly classified into obese and lean types. Obese individuals contain more *Firmicutes*, whereas lean individuals often have more *Bacteroides* (107). A typical Western diet, which is high in fat, sugar, and red meat, increases the number of obese *Firmicutes*. In contrast, a high-fiber diet rich in vegetables and fruits, such as the Japanese diet, the Mediterranean diet, the Five-a-Day diet in the USA, and vegetarian and vegan diets, increases *Bacteroidetes* (108). The Mediterranean diet, a representative healthy diet, is characterized by low intake of sweets and red meat, daily consumption of whole grains with a low glycemic index (GI), extra virgin olive oil, and approximately one glass of red wine per day. Additionally, daily physical activity is recommended as part of the Mediterranean diet pyramid.

The Japanese diet is also characterized by a high intake of foods that maintain a healthy gut microbiota, including low fat intake, high fish protein, and fermented foods (109). On the other hand, it is interesting to note that African villages have a primitive diet very high in dietary fiber, which maintains the diversity of the intestinal microbiota and a low *Firmicutes*/*Bacteroidetes* (F/B) ratio, which is considered healthy (110).

Figure 5 summarizes the mechanisms underlying the improvement in COVID-19 responses by phytochemicals (polyphenols and flavonoids), vitamin D, and gut microbiota. Phytochemical effects against COVID-19 via various mechanisms.

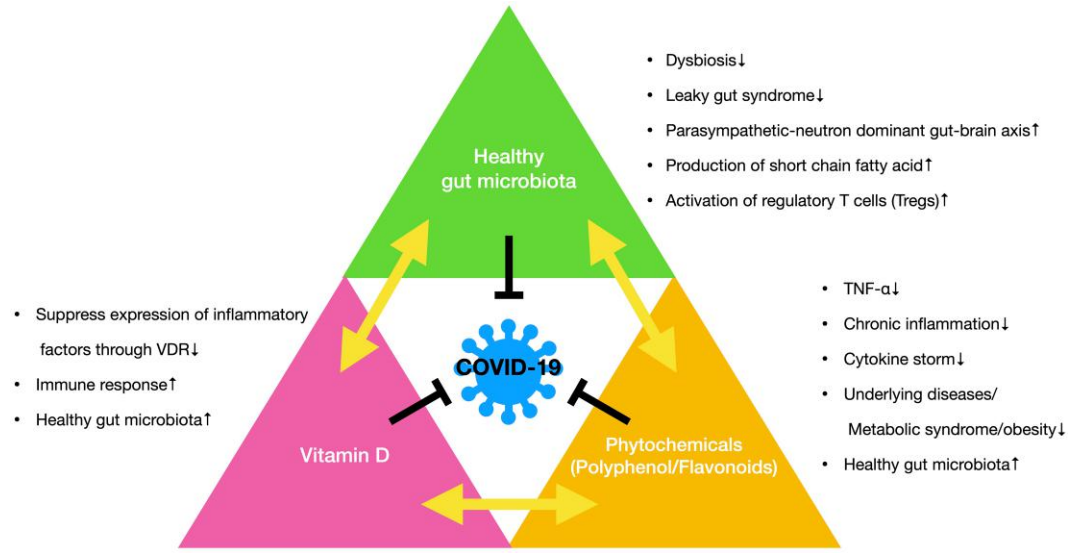

FIGURE 5
The mechanisms behind the improvement of COVID-19 responses by phytochemicals (polyphenol and flavonoids), vitamin D and gut microbiota. Phytochemicals prevent the production of proinflammatory cytokine TNF-α production; reduce chronic inflammation; prevent cytokine storms; and underlying diseases, metabolic syndrome, and obesity. Vitamin D suppresses the expression of inflammatory factors through vitamin D receptors (VDRs); upregulates immune responses; and maintains of healthy gut microbiota. Healthy gut microbiota is nurtured by both phytochemicals and vitamin D. Maintenance of healthy gut microbiota prevents dysbiosis; leaky gut syndrome; parasympathetic-neuron dominant gut-brain axis; production of short fatty acids; and upregulation of regulatory T cells (Tregs). These are associated with the prevention of the onset of COVID-19.

First, phytochemicals not only prevent the production of proinflammatory cytokine TNF-α production but also prevent cytokine storms caused by COVID-19. The suppression of chronic inflammation prevents obesity and metabolic syndrome-related diseases, which are responsible for the onset of underlying diseases. The anti-viral effects of phytochemicals are well known. Furthermore, phytochemicals function as prebiotics and maintain a healthy gut microbiota.

Recent findings on vitamin D have demonstrated its effects on the upregulation of the immune system. Since most immune cells express vitamin D receptors (VDRs), vitamin D suppresses the transcription pathways of inflammation-associated genes and cytokine storms observed in COVID-19. Vitamin D deficiency has been observed in many deadly diseases, and their supplementation boosts immune responses. Furthermore, vitamin D intake is associated with the maintenance of healthy gut microbiota.

Maintenance of a healthy gut microbiota is associated with systemic health conditions. The onset of COVID-19 is associated with dysbiosis. This induces leaky gut syndrome, which enables the penetration of bacteria and their toxins into the bloodstream and circulation around the body, thereby inducing inflammation. Malfunction of the intestine is one of the chief symptoms of COVID-19 that worsens the condition of patients. Furthermore, the gut microbiota is associated with the maintenance of the gut-brain axis and induces a parasympathetic-neuron-dominant state related to stress reduction. In addition, short fatty acids produced by the gut microbiota activate regulatory T cells (Tregs) and prevent the manifestation of symptoms even after infection with SARS-CoV-2.

Acute pneumonia due to COVID-19 resembles sepsis caused by various infections and viruses (111). In COVID-19, SARS-CoV-2 infection causes inflammation, primarily in the lower respiratory tract, and disseminated intravascular coagulation (DIC) occurs when a cytokine storm spreads throughout the body, leading to severe symptoms and death. The mechanism is an inflammatory response, such as septic shock, triggered by infections and not just viruses. Long-term COVID-19 continues to pose a problem (112). This condition is caused by an inflammatory response that affects various parts of the body, including nerve cells, resulting in an increase in the number of aging cells. The challenge in treating long COVID, as to sepsis, is the removal of senescent cells. The antimicrobial peptide LL-37 (113), which activates innate immunity, and K-FGF (114, 115), a functional food containing phytochemicals produced from Japanese grapes (fermented grape food from Koshu), are effective in this regard.

A limitation of this study is that the medical systems in developed countries such as Japan and Africa are very different, making it difficult to determine how well recorded figures capture the actual situation. Japan has also experienced a collapse in medical systems owing to COVID-19, such as a shortage of ambulances in Tokyo; however, the medical system has been well developed. By contrast, in Africa, the population with access to hospitals is much more limited. Some reports have indicated the possibility of underestimating the impact of COVID in Africa (116). The most conceivable reason derived from the serosurveillance data is significant underdetection and underreporting (117). However, it is possible that these phenomena are applicable only to limited areas, including conflict zones (118). Second, Japan has a long life expectancy and a declining population, while in Africa, the population continues to grow and there are many children, creating completely different population pyramids. Furthermore, ACE2, the receptor for SARS-CoV-2

infection, is less expressed in young people (119), and Africans have many genetic polymorphisms, the frequency of which differs from that of people in other regions (120).

Regardless of these differences, it is necessary to consider the possibility that directly applying findings obtained from one region to another may be difficult. Moreover, with predicted future developments, healthy features such as high blood vitamin D levels and diverse gut microbiota in Africa may be lost.

Notably, in this study, COVID-19 deaths in Africa were unexpectedly low, accounting for only 2% of the global deaths. This low mortality rate is attributed not only to the high proportion of children in the population, but also to the relatively low number of people with underlying metabolic and obesity-related diseases, which are mainly caused by overeating. Additionally, high average blood vitamin D levels and, more notably, a low *Firmicutes*/*Bacteroidetes* (F/B) ratio and a highly diverse gut microbiota are contributing factors. These factors may explain the lower incidence of COVID-19 and less severe disease outcomes in Africa than in developed and emerging countries. These results are indeed very interesting.

The authors have already shown that a healthy diet containing nutrients such as phytochemicals and vitamin D is associated with a healthy gut microbiota. In this context, the present study, based on an article review, shows that phytochemicals and vitamin D are involved in the improvement of COVID-19 and its sequelae by maintaining a healthy gut microbiota. Further epidemiological studies are required to confirm these findings and explore the potential of dietary interventions to mitigate the impact of COVID-19 and improve overall public health.

5 Conclusion

A comparison of the Japanese and African COVID-19 responses confirmed the importance of a healthy diet. Vitamin D is related to vitamins, and its deficiency threatens the health of the body. However, it is now recognized as an immune-related hormone. Phytochemicals have also become attractive as the seventh most important nutritional source for a healthy diet in recent years. Maintaining adequate blood vitamin D levels and taking phytochemicals are associated with maintaining a healthy and diverse gut microbiota and upregulation of immune responses, which are correlated with a low mortality rate from COVID-19. This study suggests that healthy dietary patterns and nutrients are important long-term protective factors against lung diseases, including COVID-19, and may also help prevent other diseases such as sepsis caused by infections. Promoting a diet rich in phytochemicals and ensuring sufficient vitamin D intake could serve as effective strategies to enhance public health and mitigate the global impact of infectious diseases.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000062073.

Ethics statement

Ethical approval was not required for the studies involving humans because all data used are publicly available. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements because all data used are publicly available.

Author contributions

KS: Writing – original draft, Writing – review & editing. RT: Writing – original draft, Writing – review & editing. KW: Writing – original draft, Writing – review & editing. IN: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1465324/full#supplementary-material

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Cardiometabolic factors and vitamin D deficiency in pediatric patients with chronic kidney disease

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Background: Patients with chronic kidney disease (CKD) are at increased risk for cardiovascular disease. Up to 80% of patients with CKD may exhibit inadequate vitamin D (VD) levels, which have been linked to the presence of cardiometabolic factors (CFs) in the adult population. However, research on this association in the pediatric population is limited.

Objective: To analyze the effects of 25-hydroxyvitamin D3 (25-[OH]D) levels and status on the presence of CFs in children receiving kidney replacement therapy (KRT).

Materials and methods: This cross-sectional study included pediatric patients receiving KRT, aged 8–17 years, who were receiving hemodialysis or peritoneal dialysis from January 2021 to March 2024. We conducted anthropometric measurements, blood pressure assessments, and glucose, 25-(OH)D, and lipid profiling for all participants. The daily dose of cholecalciferol supplementation, as well as other medications affecting bone and lipid metabolism and antihypertensive drugs, were documented. Statistical analyses were performed using Student's *t*-tests and chi-square tests to compare the CFs between groups with and without VD deficiency.

Results: The study involved 156 patients with an average age of 12.9 years and a mean serum VD level of 22.5 ng/dL. Patients with VD deficiency presented higher levels of total cholesterol and diastolic blood pressure ($p < 0.05$). No statistically significant differences were found in other biochemical profile variables or in the frequency of cardiometabolic factors.

Conclusion: Vitamin D deficiency seems to increase the risk of dyslipidemia and uncontrolled hypertension in children and adolescents with end-stage CKD.

KEYWORDS

dyslipidemia, vitamin D, chronic kidney disease, pediatric, cardiometabolic factors

1 Introduction

Vitamin D (VD) is a lipid-soluble steroid hormone equipped with a distinct cytosolic receptor. While initially associated with the metabolism of calcium and phosphorus, recent discoveries highlight its broader role in affecting multiple key extraskeletal systems across various target organs, including fat tissues, blood cells, immune components, skin, muscles, the pancreatic endocrine system, and vascular structures (1, 2). The VD receptor (VDR) is found in virtually all body organs and functions through both genomic (nuclear VDR) and nongenomic (membrane VDR) mechanisms. The majority of human VD is derived from the synthesis in the skin induced by sunlight (approximately 80%), with the remainder obtained from dietary intake and supplements (3, 4). The factors contributing to VD deficiency include dark skin, inactive lifestyles, inadequate sun exposure, environmental pollution, obesity, and insufficient VD supplementation (5).

In the context of chronic kidney disease (CKD)-related mineral and bone disorders, VD is crucial because of the presence of 1-α hydroxylase in the kidneys, which is pivotal for bone formation and resorption. Deficient levels of 25-hydroxyvitamin D3 (25-[OH]D) in the serum can lead to a negative calcium balance, induce secondary hyperparathyroidism, and result in bone disorders. In CKD, the increase in hyperphosphaturic osteocyte-derived hormone (FGM-23) acts to counter phosphate retention, which in turn suppresses renal 1α-hydroxylase expression and promotes 24-hydroxylase expression and results in the degradation of 1,25(OH)D. Impaired 25(OH)D absorption due to kidney disease is the predominant cause of 1,25(OH)D deficiency (3). Consequently, patients with end-stage CKD often lack both activated and nutritional VD (6).

Considering that a serum 25(OH)D level < 20 ng/mL signifies a deficiency and that >30 ng/mL is necessary for optimal health, both the Kidney Disease Outcomes Quality Initiative (KDOQI) and Kidney Disease Improving Global Outcomes (KDIGO) suggest annual testing of 25(OH)D levels for children with CKD stages 2–5 and advocate for supplementation when levels fall below 30 ng/mL (7). Additionally, the KDOQI guidelines recommend the use of cholecalciferol to address VD insufficiency in CKD stages 3 and 4 and active VD hormone treatments for VD deficiency in patients with stage 5 CKD who also exhibit secondary hyperparathyroidism, with observations showing that 25(OH)D inadequacy continues as CKD progresses from stages 3 to 5 (8).

Cardiovascular deaths in adults with CKD are due primarily to coronary artery disease and congestive heart failure. In contrast, the leading causes of death within the pediatric population include arrhythmias, valvular diseases, cardiomyopathy, and cardiac arrest. This distinction highlights the varying impact of cardiovascular complications across different age groups with CKD (9, 10).

Endothelial dysfunction, an early indicator of cardiac issues, manifests in the initial stages of CKD in both children and adults (9). Recent studies focusing on the pediatric population have revealed a high incidence and prevalence of cardiovascular risk factors associated with CKD. These findings underscore the importance of early detection and management of endothelial health in young patients to mitigate long-term cardiovascular complications (11).

Abbreviations: 25-[OH]D, 25-hydroxyvitamin D3; CKD, chronic kidney disease; VD, vitamin D; VDR, vitamin D receptor; FGF-23, osteocyte-derived hormone; CFs, cardiometabolic factors.

Previous studies in adults have demonstrated significant associations between vitamin D deficiency and an increased risk of cardiometabolic factors (CFs), including metabolic syndrome. For example, among adult patients on hemodialysis, the prevalence of metabolic syndrome increased as vitamin D levels decreased, with the highest prevalence observed in those with 25(OH)D levels below 20 ng/mL. These patients also exhibited negative associations between vitamin D and factors such as diastolic blood pressure and triglyceride levels (12). Further studies indicate that low levels of 25(OH)D are associated with components of metabolic syndrome, such as central obesity, hypertension, and dyslipidemia (13). In contrast, no significant associations between 25(OH)D and metabolic syndrome were observed in patients on peritoneal dialysis, suggesting possible differences related to the type of CKD treatment (14). Additionally, the implications of elevated parathyroid hormone (PTH) and its relationship with lipogenesis and obesity are discussed (15).

Clinical trials and meta-analyses have shown a potentially beneficial effect of vitamin D supplementation on reducing total serum cholesterol, LDL cholesterol, and triglyceride levels in patients with hypercholesterolemia and vitamin D insufficiency (16, 17).

Research in the pediatric population suggests an association between vitamin D deficiency and risk factors for metabolic syndrome, including increased HDL cholesterol and the mitigation of triglyceride increases (18). Specifically, 2,596 students with a mean age of 12.2 years were studied, and it was demonstrated that those with deficient vitamin D levels had higher odds of Metabolic Syndrome (OR: 4.25), abdominal obesity (OR: 2.24), low HDL-C (OR: 1.65), and high fasting blood sugar (OR: 2.56) compared to those with sufficient vitamin D levels (19). Recent studies have shown an association between vitamin D deficiency and cardiovascular diseases during the progression of CKD (20). A study of 34 children with CKD revealed that vitamin D levels are inversely correlated with increases in the left ventricular mass index ($r = -0.5$; $p < 0.05$) (21).

These findings suggest that the relationship between vitamin D and CFs may vary depending on the population and the status of kidney disease, highlighting the need to explore these links in children with CKD to better understand their impact on this young population.

Therefore, our study aimed to analyze the effects of the level and status of 25-hydroxyvitamin D3 (25-[OH]D) on the presence of CFs in children with CKD.

2 Materials and methods

2.1 Subjects

This cross-sectional study was conducted from January 2021 to March 2024 at two tertiary pediatric care centers in Mexico City. We enrolled children aged 6 to 18 years who were diagnosed with stage 5 CKD, as classified by the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) (22). These children were receiving replacement therapy with either peritoneal dialysis or hemodialysis. Patients with a family history of dyslipidemia, hepatic insufficiency, or those receiving steroid therapy were excluded from the study.

Among the 169 potential candidates for inclusion, 13 were excluded (5 children were younger than 6 years, 3 underwent kidney transplants less than 3 months previously, and 11 declined to

participate in the study). The final number of participants included was 156 patients.

In accordance with the Declaration of Helsinki, the protocol was evaluated and approved by the ethics and research committee of the hospital with registry numbers R-2010-3603-7, R-2023-785-096, and HIM-2017-117. The parent or legal guardian signed a written informed consent form, and each child provided written assent according to the recommendations of the Declaration of Helsinki.

2.2 Variables

The patient records provided demographic data, the type of renal replacement therapy (dialysis or hemodialysis), the etiology of chronic kidney disease (CKD), duration since diagnosis, diagnosis of hypertension, cholecalciferol supplementation and dosage, and use of calcitriol, lipid-lowering agents, phosphate binders, calcimimetics, and antihypertensives. To determine and quantify the adequacy of hemodialysis and peritoneal dialysis treatments, the Kt/V was calculated (K, dialyzer clearance of urea; t, dialysis time; V, volume of distribution of urea). Hemodialysis was deemed adequate when Kt/V exceeded 1.2 per week, and peritoneal dialysis was considered adequate when Kt/V was greater than 1.8 per week (22, 23).

2.2.1 Anthropometry and blood pressure

The anthropometric indicators of each patient were recorded by a certified nutritionist. Height was measured to the nearest 0.1 cm with a SECA Model 769 stadiometer (SECA 769, SECA Corp. Oakland Center Columbia, MD, United States). Weight measurements were conducted using the bioimpedance method (Tanita BC-568 Segmental Body Composition Monitor, Tokyo, Japan), with patients barefoot and wearing underwear. Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared, and then the percentile and BMI z score were obtained according to age and sex. Classification of BMI was defined by the Centers for Disease Control and Prevention 2000, with children considered obese when their BMI for age and sex was in the ≥95th percentile, overweight when their BMI was >85th but <95th percentile, malnourished when their BMI was < 25th percentile, and normal weight when their BMI was within the 25th and 84th percentiles (24). Blood pressure was measured with auscultatory methods using a mercury sphygmomanometer according to age in duplicate and reported as a percentile according to age, sex, and height. Measurements were taken with the participant sitting in a chair with their feet flat on the floor and their back supported after a 10-min rest period in the hospital (25, 26).

2.2.2 Blood analysis

Blood samples were obtained from the forearm of each subject via the antecubital vein between 7:00 and 8:00 a.m., and after a minimum of 12 h of fasting. Serum samples were frozen at −80°C until analysis. Glucose, triglycerides (TGLs), high-density lipoprotein cholesterol (HDLc), urea, creatinine, and parathyroid hormone levels were determined by colorimetric enzymatic methods (Bayer Diagnostics, Puteaux, France). Intra- and interassay coefficients of variation <7% were considered acceptable. A standard curve was also generated for each assay. For LDL-C, we utilized DeLong's modified Friedwald formula (24).

2.2.3 Cardiometabolic profile (definition)

Children with diastolic or systolic blood pressure ≥ the 90th percentile for age and sex, according to the National Blood Pressure Education Program Working Group were considered to have hypertension (26). Obesity was indicated by a BMI ≥ the 95th percentile, and overweight was indicated by a BMI ≥ the 85th percentile for age and sex according to the 2000 CDC Growth Charts (27). For patients whose height was more than 2 standard deviations less than average for age, the BMI z score was adjusted for height and age. A fasting glucose level ≥ 100 mg/dL was considered elevated (27). Hypertriglyceridemia was assessed TGLs ≥90th percentile for age and sex for children <10 years old and TGLs ≥150 mg/dL for children >10 years old (27, 28). LDL hypercholesterolemia was assessed as LDLc ≥90th percentile for age and sex for children <10 years old and LDLc ≥130 mg/dL for children >10 years old. Reduced HDLc was defined as HDLc <10th percentile for age and sex for children <10 years old and HDLc <40 mg/dL in males and < 50 mg/dL in females for children >10 years old, according to the International Diabetes Federation (IDF) definition (27, 28). Dyslipidemia was defined as the presence of hypertriglyceridemia, reduced HDLc or LDL hypercholesterolemia. Hypertension was assessed using systolic and diastolic blood pressure according to age. In patients <13 years, systolic or diastolic blood pressure ≥ 95th percentile for age, height, and sex was considered hypertensive. For those >13 years old, those whose systolic blood pressure was ≥130 mmHg or whose diastolic blood pressure was ≥80 mmHg (29) were considered hypertensive if their medical records indicated a diagnosis and if they were taking antihypertensive medication to manage it. Cases of uncontrolled hypertension were identified when, despite the use of antihypertensives, physical examinations revealed elevated blood pressure levels based on the previously mentioned criteria.

2.3 Vitamin D determination

The serum concentrations of 25(OH)D (25-hydroxyvitamin D3) were measured using the Abbot chemoluminescence technique with Archirech 1,000 equipment. A serum level of <20 ng/mL was considered VD deficiency, 20–29.99 ng/mL was considered insufficient, and > 30 ng/mL was considered normal (30).

2.4 Statistical analyses

For quantitative variables, Kolmogorov–Smirnov tests were performed to evaluate the normality of the distribution. The quantitative variables were not normally distributed, so they are presented as the means and standard errors. The qualitative variables are presented as proportions and frequencies. While the Endocrine Society classifies vitamin D status into three categories (deficiency <20 ng/mL, insufficiency 20–29.99 ng/mL, and normal >30 ng/mL) (31). Initially, the patients were compared across these three groups, but no statistically significant differences were found in the multivariate models. Studies that have found links between vitamin D levels and cardiometabolic factors often use a two-group classification: deficiency (<20 ng/mL) and no deficiency (>20 ng/mL) (17, 32). Accordingly, this study grouped patients into two categories: those with vitamin D deficiency and those without. A sub-analysis was

conducted according to age groups: school-aged children (6–10 years, $n = 36$) and adolescents (11–18 years, $n = 120$). Logarithmic transformation of the quantitative variables was performed for statistical tests. The comparisons of qualitative variables between the groups were performed using the chi-square test and the Student's *t*-test was used for quantitative variables. To evaluate the correlation of biochemical parameters with serum VD levels, the Spearman test was used. A multiple linear regression model was used to evaluate the association of serum vitamin D levels with LDL cholesterol and adjusted for confounding variables including age, sex, body mass index z score, cholecalciferol supplementation dose, and replacement treatment. A multiple logistic regression model was used to evaluate the association of vitamin D deficiency with the presence of dyslipidemia and uncontrolled hypertension with adjustments for confounding variables (age, parathyroid hormone, obesity, and cholecalciferol supplementation dose). The model was constructed considering subjects who had one or more cardiometabolic factors (dyslipidemia and hypertension) in order to make the model more robust. A value of $p < 0.05$ was considered statistically significant. STATA software (Stata Corp, College Station, TX, United States), version 12.0, was used for the statistical analyses.

3 Results

Of the 156 participants, the average age was 12.9 years, 51.9% were female, and 78.8% were classified as normal weight based on their BMI z score. The most common etiology of CKD was congenital anomalies of the kidney and urinary tract (CAKUT), which were observed in 47.4% of cases. The modalities of renal replacement therapy, peritoneal dialysis, and hemodialysis were distributed in similar proportions, with only 10.9% showing inadequate dialysis (Table 1). The average level of 25-hydroxyvitamin D was 22.5 ng/dL. Vitamin D deficiency was present in 21.2% ($n = 33$) of the patients, whereas nearly half (48.7%, $n = 76$) had sufficient levels of VD (Table 1).

Table 2 presents a comparison of lipid profiles and cardiometabolic factors according to whether children had vitamin D deficiency (Table 2). In terms of the biochemical profile, the average glucose level was 90 mg/dL, and the total cholesterol level was 162.9 mg/dL. The most frequent cardiometabolic alterations related to the biochemical profile were hypertriglyceridemia (42.9%, $n = 67$) and low HDL cholesterol levels (42.3%, $n = 66$), with 66.7% of the patients presenting with dyslipidemia. Additionally, 57.0% ($n = 89$) of the patients had hypertension, 53 of whom had uncontrolled hypertension.

A comparison of the biochemical profiles of patients with VD deficiency ($n = 76$) and without ($n = 80$) VD deficiency revealed that those with deficiency presented higher total cholesterol levels (174.5 mg/dL vs. 151.9 mg/dL, $p = 0.001$), LDL cholesterol levels (163.9 mg/dL vs. 148.2 mg/dL, $p = 0.006$), and diastolic blood pressure (74.4 mmHg vs. 70.4 mmHg, $p = 0.033$). When comparing the lipid profile and cardiometabolic alterations between patients with and without vitamin D deficiency across different age groups, it was observed that both school-aged children and adolescents with vitamin D deficiency had higher total cholesterol levels compared to those without the deficiency. As for hypertriglyceridemia, a higher proportion was found only in vitamin D-deficient school-aged children (80.0% vs. 42.9%) (Table 3).

TABLE 1 Characteristics of pediatric patients with chronic kidney disease included in the study.

Characteristics	Total $n = 156$
Age, y	
Mean (Standard Error)	12.9 (0.25)
Sex, %	
Female	81 (51.9)
Male	75 (48.1)
Anthropometry, mean (standard error)	
Weight, kg	35.6 (1.07)
Height, cm	139.2 (1.61)
Height-for-age z score	−2.6 (0.14)
Body mass index, kg/m^2	17.6 (0.27)
Body mass index z score	−0.93 (0.11)
Nutritional status, n (%)	
Normal (25th and 84th pc)	123 (78.8)
Malnutrition (< 25th pc)	27 (10.9)
Overweight/obesity (≥85th pc)	16 (10.3)
CKD etiology, n (%)	
CAKUT 4	74 (47.4)
Glomerulopathy	33 (21.2)
Tubulopathy	2 (1.3)
Immunological	6 (3.8)
Indeterminate	41 (26.3)
Replacement treatment; n (%)	
Hemodialysis	75 (48.1)
Peritoneal dialysis	81 (51.9)
Time of renal replacement, y	
Mean (Standard Error)	3.8 (0.28)
Kt/V altered, n (%)	
Yes	17 (10.9)
25(OH)D, ng/mL	
Mean (Standard Error)	22.5 (0.97)
Vitamin D status, %	
<20 ng/mL (deficiency)	76 (48.7)
20–29.9 ng/mL (insufficiency)	47 (30.1)
≥30 ng/mL (normal)	33 (21.2)

25(OH)D, 25-hydroxyvitamin D3; CAKUT, congenital anomalies of the kidney and the urinary tract; CKD, chronic kidney disease.

The serum concentrations of each biochemical parameter were correlated with the serum vitamin D concentration, revealing a weak negative correlation with total cholesterol and LDL cholesterol ($p < 0.05$). When performing the sub-analysis of the correlation according to age group, the correlations with total cholesterol and LDL cholesterol remained consistent (Table 4).

The frequency of cardiometabolic factors such as dyslipidemia and uncontrolled hypertension was also significantly greater in

TABLE 2 Comparison of lipid profiles and cardiometabolic factors according to vitamin D deficiency and sufficiency in pediatric patients with chronic kidney disease (CKD) included in the study.

Characteristics	All $n = 156$	Vitamin D deficiency		p*
		With $n = 76$	Without $n = 80$	
Biochemical profile and blood pressure, mean (standard error)				
Glucose, mg/dL	90.0 (1.58)	91 0.6 (2.40)	88.5 (2.07)	0.162
Total cholesterol, mg/dL	162.9 (3.79)	174.5 (6.48)	151.9 (3.73)	*0.001*
HDL cholesterol, mg/dL	47.7 (1.11)	47.6 (1.55)	47.8 (1.60)	0.467
LDL cholesterol, mg/dL	82.9 (3.01)	90.6 (4.89)	75.6 (3.41)	*0.006*
Triglycerides, mg/dL	155.8 (6.46)	163.9 (9.66)	148.2 (8.61)	0.112
Systolic blood pressure, mmHg	112.6(1.33)	112.8 (1.79)	112.3 (1.97)	0.423
Diastolic blood pressure, mmHg	72.4 (1.08)	74.4 (1.50)	70.4 (1.53)	*0.033*
Parathormone, pg./mL	629.1 (50.3)	638.1 (70.0)	620.6 (72.5)	0.431
Cardiometabolic factors, n (%)				
Hyperglycemia	33 (21.1)	18 (23.7)	15 (18.7)	0.451
Decreased HDLc	66 (42.3)	37 (48.7)	29 (36.2)	0.116
LDL hypercholesterolemia	13 (8.3)	8 (10.5)	5 (6.2)	0.334
Hypertriglyceridemia	67 (42.9)	35 (46.1)	32 (40.0)	0.445
Dyslipidemia	104 (66.7)	56 (73.7)	48 (60.0)	0.070
Hypertension	89 (57.0)	48 (63.2)	41 (51.2)	0.133
Uncontrolled hypertension	53 (34.0)	31 (40.8)	22 (27.5)	0.080
Dyslipidemia or uncontrolled hypertension	118 (75.6)	63 (82.9)	55 (68.7)	*0.040*
Confounding factors, n (%)				
Hyperparathyroidism	92 (59.0)	49 (64.5)	43 (53.7)	0.173
Use of phosphate binders	112 (73.2)	58 (76.3)	56 (70.0)	0.374
Use of cholecalciferol supplementation	79 (50.6)	24 (31.6)	55 (68.7)	*0.001*
Cholecalciferol supplementation dose, UI, mean (standard error)	982.0 (112.2)	168.4 (41.0)	1755.0 (176.4)	*0.001*
Use of calcitriol	82 (73.9)	48 (77.4)	34 (69.4)	0.339
Use of lipid-lowering agents	25 (16.0)	15 (19.7)	10 (12.5)	0.218
Obesity	11 (7.0)	4 (5.3)	7 (8.7)	0.395

*Student's t-test for independent data and chi^2 Pearson.
The values in italics indicate a significance level of $p < 0.05$.

patients with VD deficiency (82.9% versus 68.7%, $p = 0.040$). The use of cholecalciferol supplementation was greater in patients without vitamin D deficiency (68.7% vs. 31.6%, $p = 0.001$), as were the daily doses they consumed (1755.0 IU/day vs. 168.4 IU/day, $p = 0.001$). There were no differences in the effects of the other medications on bone metabolism between patients with and without VD deficiency.

Regarding the use of drugs that affect bone metabolism, 73.2% used phosphate binders (calcium carbonate, sevelamer), 50.6% used cholecalciferol with an average daily dose of 982.0 IU, and 73.9% consumed calcitriol at doses of 0.15–0.25 mcg/kg/day, with none using calcimimetics. Nearly two-thirds of the patients suffered from hyperparathyroidism (59.0%) (Table 2).

In the multivariate linear analysis, a negative association was found between VD levels (B −0.600, 95% CI −1.109 to −0.090), and a positive association was detected between peritoneal dialysis (7.751, 95% CI 1.007–14.495) and LDL cholesterol concentrations, controlling for age, sex, BMI z score, parathyroid hormone levels, and cholecalciferol supplementation (Table 5). The multiple logistic model showed that vitamin D deficiency is associated with an increased risk of dyslipidemia and uncontrolled hypertension (OR 2.2, 95% CI 1.1–4.8) when adjusted for age, obesity, parathyroid hormone levels, and cholecalciferol supplementation (Table 6).

4 Discussion

In the study involving patients with CKD, VD deficiency and insufficiency were observed in half of the participants. This prevalence was lower than that typically reported in this population, where rates range from 62.5% to over 80% (32, 33).

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TABLE 3 Comparison of lipid profiles and cardiometabolic factors between patients with vitamin D deficiency and sufficiency according to age group in pediatric patients with chronic kidney disease (CKD) included in the study.

Characteristics	Vitamin D deficiency		*p**	Vitamin D deficiency		*p**
	With *n* = 15	Without *n* = 21		With *n* = 61	Without *n* = 59	
Biochemical profile and blood pressure, mean (standard error)	School-aged children (6–10 y) (*n* = 36)			Adolescents (11–18y) (*n* = 120)		
Glucose, mg/dL	100.7 (7.62)	89.2 (4.19)	0.077	89.2 (2.28)	88.2 (2.40)	0.579
Total cholesterol, mg/dL	213.3 (6.48)	165.1 (8.46)	*0.015*	*165.0 (6.33)*	*147.1 (3.93)*	*0.041*
HDL cholesterol, mg/dL	50.5 (3.19)	50.1 (3.91)	0.961	46.9 (1.68)	47.0 (1.85)	0.674
LDL cholesterol, mg/dL	118.2 (16.08)	83.9 (7.42)	0.112	83.8 (4.31)	72.6 (3.76)	0.068
Triglycerides, mg/dL	214.3 (20.29)	158.6 (17.26)	0.044	151.5 (10.4)	144.5 (9.98)	0.569
Systolic blood pressure, mmHg	103.2 (3.63)	104.5 (4.18)	0.834	115.2 (|0.94)	115.1 (2.14)	0.769
Diastolic blood pressure, mmHg	68.3 (2.46)	64.6 (3.01)	0.628	75.9 (1.72)	72.5 (1.71)	0.200
Parathormone, pg./mL	517.0 (168.0)	650.4 (151.6)	0.596	667.8 (77.1)	610.0 (83.0)	0.280
Cardiometabolic factors, n (%)						
Hyperglycemia	5 (33.3)	7 (33.3)	1.000	13 (21.3)	8 (13.6)	0.264
Decreased HDLc	5 (33.3)	7 (33.3)	1.000	31 (52.5)	22 (37.3)	0.095
LDL hypercholesterolemia	4 (26.7)	2 (9.5)	0.174	4 (6.6)	3 (5.1)	0.731
Hypertriglyceridemia	*12 (80.0)*	*9 (42.9)*	*0.026*	23 (37.7)	23 (38.9)	0.886
Dyslipidemia	13 (86.7)	13 (61.9)	0.102	43 (70.5)	35 (59.3)	0.200
Hypertension	8 (53.3)	11 (52.4)	0.955	40 (65.6)	30 (50.8)	0.102
Uncontrolled hypertension	6 (40.0)	5 (23.8)	0.298	25 (41.0)	17 (28.8)	0.162
Dyslipidemia or uncontrolled hypertension	15 (71.4)	13 (86.7)	0.278	50 (82.0)	40 (67.8)	0.073
Confounding factors, n (%)						
Hyperparathyroidism	7 (46.7)	12 (57.1)	0.535	42 (68.8)	31 (52.5)	0.067
Use of phosphate binders	12 (80.0)	17 (80.9)	0.943	46 (75.4)	39 (66.1)	0.262
Use of cholecalciferol supplementation	8 (53.3)	14 (66.7)	0.418	*16 (26.2)*	*41 (69.5)*	*<0.001*
Cholecalciferol supplementation dose, UI, mean (standard error)	*168.4 (41.0)*	*1755.0 (176.4)*	*0.035*	*157.3 (48.8)*	*1796.6 (205.6)*	*<0.001*
Use of calcitriol	12 (80.0)	13 (61.9)	0.245	43 (70.5)	33 (55.9)	0.098
Use of lipid-lowering agents	3 (20.0)	4 (19.0)	0.943	12 (19.7)	6 (10.2)	0.145
Obesity	2 (9.5)	1 (6.7)	0.760	3 (4.9)	5 (8.5)	0.435

The values in italics indicate a significance level of $p < 0.05$.

VD deficiency in patients with CKD is attributed to multiple factors, including dietary restrictions and reduced cutaneous synthesis of vitamin D due to uremia. Moreover, as renal function declines, there is a decrease in megalin, an increase in phosphate and FGF-23, and abnormalities in PTH and the retention of uremic toxins that inhibit 1α-hydroxylase activity (34, 35). This leads to reduced serum levels of calcidiol (25[OH]D) and decreased conversion to calcitriol (1,25-OH-D), causing disturbances in bone metabolism and secondary hyperparathyroidism, which was present in our patients (36).

VD is linked to cholesterol metabolism, as both share a common biosynthetic pathway. Calcitriol, the active form of VD, plays roles in remodeling and regulating inflammatory processes that drive atherosclerosis, including the proliferation of smooth muscle cells, which stabilize plaques. Calcitriol impedes cholesterol absorption by macrophages and promotes cholesterol efflux, suggesting that vitamin D metabolites may suppress foam cell formation and, consequently, atherosclerosis itself. Some interactions observed between vitamin D metabolism and cholesterol can be explained by calcidiol (25[OH]D) suppressing the activity of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. Moreover, the vitamin D receptor induces the activity of the enzyme cholesterol 7 alpha-hydroxylase (CYP7A1), which is responsible for converting cholesterol into 7a-hydroxy cholesterol, a bile acid precursor. Specifically, regarding LDL cholesterol, VD deficiency is associated with elevated total cholesterol and LDL-C levels, and supplementation with VD could decrease total serum cholesterol and LDL-C levels (37, 38).

TABLE 4 Correlation between levels of 25-hydroxyvitamin D3 and biochemical profile, blood pressure and parathormone in pediatric patients with chronic kidney disease (CKD).

	All ($n = 156$)		School-aged children (6–10 y) ($n = 36$)		Adolescents (11–18 y) ($n = 120$)	
Characteristics	*r*	*p**	*r*	*p**	*r*	*p**
Glucose, mg/dL	−0.044	0.583	−0.183	0.284	0.004	0.961
Total cholesterol, mg/dL	*−0.264*	*0.001*	*−0.385*	*0.020*	*−0.260*	*0.004*
HDL cholesterol, mg/dL	−0.113	0.160	0.0001	0.999	−0.168	0.065
LDL cholesterol, mg/dL	*−0.212*	*0.007*	*−0.293*	*0.049*	*−0.214*	*0.018*
Triglycerides, mg/dL	−0.107	0.181	−0.274	0.104	−0.072	0.429
Systolic blood pressure, mmHg	0.049	0.536	−0.043	0.802	0.124	0.176
Diastolic blood pressure, mmHg	−0.068	0.393	−0.239	0.159	0.012	0.896
Parathormone, pg./mL	0.004	0.959	0.073	0.668	−0.018	0.844

*Spearman correlation.
The values in italics indicate a significance level of $p < 0.05$.

TABLE 5 Effect of vitamin D concentrations on LDL cholesterol levels in pediatric patients with chronic kidney disease (CKD) included in the study.

Characteristics	Coefficient	CI 95%	*p**
25(OH)D, ng/mL	−0.600	−1.109 to −0.090	*0.021*
Age, y	−2.613	−4.422 to −0.804	*0.005*
Male sex	6.435	−5.139 to 18.009	0.274
Body mass index, z score	1.009	−3.242 to 5.262	0.640
Cholecalciferol supplementation dose, UI	0.001	−0.004 to 0.006	0.770
Peritoneal dialysis	7.751	1.007 to 14.495	*0.005*

*Multiple linear regression model adjusted. R-squared = 0.135, $p = 0.001$.
The values in italics indicate a significance level of $p < 0.05$.

TABLE 6 Association of vitamin D deficiency on the presence of dyslipidemia or uncontrolled hypertension in pediatric patients with chronic kidney disease (CKD) included in the study.

Characteristics	OR	CI 95%	*p**
Vitamin D deficiency	2.212	1.007–4.856	*0.048*
Age, y	1.021	0.907–1.149	0.723
Paratohormone, pg./mL	1.000	0.999–1.001	0.057
Obesity (>95th PC)	4.865	0.560–42.236	0.151
Cholecalciferol supplementation dose, UI	0.999	0.999–1.000	0.114

*Multiple logistic regression model adjusted. R-squared = 0.063, $p = 0.026$.
The values in italics indicate a significance level of $p < 0.05$.

Building on these findings, our study observed that patients with VD deficiency presented higher levels of LDL cholesterol (163.9 mg/dL vs. 148.2 mg/dL, $p = 0.006$) than did those without VD deficiency. While similar studies in pediatric patients with CKD are lacking, research in the adult CKD population has noted that total serum cholesterol levels are higher in patients with VD deficiency than in those without deficiency (158.0 mg/dL vs. 128.6 mg/dL, $p = 0.001$). Furthermore, a two-year follow-up revealed increased mortality rates in the VD-deficient group (39% vs. 21%, $p = 0.03$) (39).

In addition to VD deficiency, various factors increase cardiometabolic risk in pediatric patients with CKD. The frequencies of dyslipidemia and hypertension can reach 83 and 56%, respectively (40, 41). In comparison, the frequency of these conditions in our study was similar, with 75% of patients with dyslipidemia and 57% with hypertension. Patients with CKD at any stage are prone to lipid abnormalities due to altered lipoprotein metabolism caused by decreased glomerular filtration. The rate of dyslipidemia is higher in patients on peritoneal dialysis, driven by increased lipoprotein synthesis in the liver due to glucose absorption from the dialysis solution, which increases insulin levels and protein loss (42, 43). Dyslipidemia may also arise due to the reduced activity of liver lipoprotein lipase and hepatic triglyceride lipase enzymes, which are influenced by uremic toxins and high levels of apoprotein C-III (22, 44). Hypertension in patients with CKD is commonly due to the inability of the kidneys to balance sodium, leading to excessive peripheral vasoconstriction (45), and hypervolemia, specifically leading to an increase in diastolic pressure (46). This condition is exacerbated by various factors, including a reduction in the number

of glomeruli, sclerosis, tubular atrophy, interstitial fibrosis, inappropriate nitric oxide release, high renin–angiotensin system activity, and abnormal synthesis of polyunsaturated fatty acids and eicosanoids (45, 47). Additionally, CKD is associated with reduced insulin secretion and sensitivity (48), elevated adipokine levels such as leptin (49), and other CKD-related issues such as hyperparathyroidism and an inflammatory state, which contribute to an increased risk of hyperglycemia (50).

In this study, we found that patients with VD deficiency had higher levels of diastolic blood pressure (74.4 mmHg vs. 70.4 mmHg) and a greater proportion of uncontrolled hypertension (40.8% vs. 27.5%). While these findings have been reported in other studies, they have not been documented in pediatric patients with CKD. Several key mechanisms can explain how VD deficiency may contribute to hypertension. One mechanism involves the activation of the renin-angiotensin-aldosterone system. As plasma renin levels increase, sympathetic activity may become heightened, raising intra-glomerular pressure, which leads to elevated blood pressure, reduced glomerular filtration rate, and cardiovascular damage. VD may also have a direct impact on left ventricular hypertrophy and vascular stiffness. Low serum 25(OH)D levels can reduce nitric oxide production in blood vessels and decrease calcium influx, impairing vasodilation and contributing to increased blood pressure (51–53).

In our study, we were unable to find an association between the dose of VD supplementation and serum LDL cholesterol levels through the multivariate model, despite evidence from other studies demonstrating its effectiveness in patients with dyslipidemia (17). Based on our findings, we recommend that pediatric patients with end-stage CKD routinely have their 25(OH)D levels measured and be supplemented with cholecalciferol to maintain normal levels (>30 ng/mL). While this may not completely prevent the presence of cardiometabolic alterations, it could help reduce them to some extent. Furthermore, a clinical trial is necessary to evaluate the efficacy of vitamin D supplementation in pediatric patients with CKD and VD deficiency in lowering serum LDL cholesterol levels.

In light of these findings, it is important to acknowledge that a major limitation of this study is the temporal ambiguity resulting from the simultaneous measurement of vitamin D deficiency and cardiometabolic factors. Due to the cross-sectional design, this prevents us from establishing causality in this apparent association. Moreover, for uncontrolled hypertension, there are other factors that may contribute beyond VD deficiency, such as medication adherence, volume overload, and myocardial dysfunction, which were not measured. Another limitation is the variability in the duration of cholecalciferol supplementation among patients, which likely influenced the lack of observed benefit in the regression models.

In conclusion, vitamin D deficiency seems to increase the risk of dyslipidemia and uncontrolled hypertension in children and adolescents with end-stage CKD. These findings suggest the need to monitor vitamin D levels in order to provide appropriate supplementation when levels considered to be deficient are detected.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

In accordance with the Declaration of Helsinki, the protocol was evaluated and approved by the ethics and research committee of the hospital with registry numbers R-2010-3603-7, R-2023-785-096, & HIM-2017-117. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

IP-O: Conceptualization, Project administration, Supervision, Validation, Writing – review & editing. JZ-C: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. MV-K: Supervision, Validation, Writing – review & editing. MK-K: Data curation, Validation, Writing – review & editing. JV-G: Investigation, Writing – review & editing. CZ-M: Investigation, Writing – review & editing. ÁR: Investigation, Writing – review & editing. GA-T: Investigation, Writing – review & editing. BR-N: Supervision, Writing – review & editing. JR-V: Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Implications of vitamin D levels or status for mortality in rheumatoid arthritis: analysis of 2001-2018 data from the National Health and Nutrition Examination Survey

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Background: Inadequate levels of vitamin D (VitD) have been linked to increased rates of various health conditions and mortality. However, little is known about the relationship between mortality outcomes and 25-hydroxyvitamin D [25(OH)D] levels in individuals with rheumatoid arthritis (RA). This study aimed to examine this association using data from the National Health and Nutrition Examination Survey.

Methods: A cohort of 2,290 individuals aged 20 to 85 years with RA was analyzed. Lower 25(OH)D levels were inversely associated with all-cause mortality, with a hazard ratio (HR) of 0.91 (0.87 to 0.96) per 10 nmol/L increase. Comparatively, the HR for the VitD insufficiency group was 0.64 (0.50 to 0.83), and for the VitD sufficiency group, it was 0.60 (0.44 to 0.80), both compared to the VitD deficiency group. Cause-specific analysis showed that higher 25(OH)D levels were associated with reduced mortality from heart disease (HR: 0.88, 0.82 to 0.95) and malignant neoplasms (HR: 0.86, 0.79 to 0.94). No significant correlation was found between 25(OH)D levels and cause-specific mortalities for other conditions.

Results: Stratified by gender, the HR for males was 0.92 (0.85 to 0.99) and for females was 0.91 (0.86 to 0.98) per 10 nmol/L increase in 25(OH)D levels. Among individuals aged 20-59 years, no significant correlation was observed, while for those aged 60 years and older, the HR was 0.86 (0.82 to 0.90) per 10 nmol/L increase. Nonlinear analysis identified a sharp increase in HR below 59.95 nmol/L, while HR remained below 1 for 25(OH)D levels above 59.95 nmol/L.

Conclusion: This study reveals a strong negative correlation between 25(OH)D levels and overall mortality in individuals with RA. Notably, this association is

particularly significant for mortality related to heart disease and malignant neoplasms. Targeted VitD supplementation should be emphasized, especially in individuals aged 60 years and older with RA. The proposed minimum threshold for adequate 25(OH)D levels in the RA population is 60 nmol/L.

KEYWORDS

vitamin D, vitamin D levels, vitamin D status, mortality, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by persistent inflammation primarily targeting the joints, resulting in discomfort, swelling, and impaired physical functioning. The global prevalence of RA is approximately 0.5%, with a higher susceptibility observed in women (1). Despite extensive research, the precise etiology of RA remains incompletely understood, though evidence suggests that immune system dysregulation and genetic predisposition contribute significantly to its development (2). Environmental factors such as UV exposure, air pollution, and dietary habits, as well as lifestyle choices including smoking, alcohol consumption, and physical activity levels have been implicated in the development and progression of RA (3, 4).

The association between Vitamin D (VitD) deficiency and autoimmune diseases, including RA, has garnered increased attention in recent years (5). Research conducted in various countries has consistently demonstrated a notable prevalence of VitD deficiency among individuals diagnosed with RA (6–9). Furthermore, lower levels of VitD have been found to correlate with more severe clinical manifestations of RA (10).

VitD deficiency is prevalent among RA patients across different ethnic groups and geographical regions. In the United States, a study found that 84% of African American RA patients had suboptimal VitD levels (11). Similar trends have been observed in other populations, with another study reporting that 84% of Caucasian RA patients had suboptimal VitD levels (12). The global nature of this issue is further emphasized by studies from various countries. In Saudi Arabia, a cross-sectional study revealed that among RA patients, 42.7% had insufficient VitD levels, 22.3% had a deficiency, and 15.5% had severe deficiency (7). This study also found significant correlations between VitD levels and disease activity measures. Research from Iran reported that 34.8% of RA patients had insufficient VitD levels. Importantly, this study demonstrated a significant relationship between serum VitD levels and disease severity (9). A meta-analysis of studies from India showed that 76.1% of RA patients were VitD deficient (13). These findings collectively underscore that VitD deficiency in RA patients is a global phenomenon, not limited to any particular ethnic group or geographical region.

Despite ongoing research, the precise nature of the relationship between VitD and RA remains incompletely understood, leaving uncertainty regarding whether VitD is a causative factor or an outcome of the condition (14). A recent meta-analysis failed to establish definitive evidence linking 25-hydroxyvitamin D [25(OH)D] levels with susceptibility to RA (15). However, a study conducted in China involving 493 RA patients revealed a genetic association between VitD metabolism pathway genes (CYP2R1 and CYP27B1) and the genetic background of RA. Moreover, a correlation was observed between alterations in methylation levels of VitD receptor (VDR) and CYP27B1, as well as an increased susceptibility to RA, indicating the potential involvement of dysregulated VitD metabolism in the onset of RA (16).

Over the past few years, researchers have increasingly focused on investigating the relationship between VitD and both all-cause mortality and cause-specific mortality. Serum levels of 25(OH)D have consistently demonstrated a negative correlation with all-cause mortality, as well as mortality specifically attributed to cardiovascular diseases (CVD), cancer, and respiratory system diseases (17–20). It is worth noting that individuals with RA often exhibit lower 25(OH)D levels and higher mortality rates compared to the general population (21, 22). A study conducted in Mexico revealed a significant association between insufficient 25(OH)D levels and an elevated risk of suicide in individuals with RA (23). Nonetheless, there is currently a lack of published studies investigating the relationship between mortality and 25(OH)D levels within the RA population.

The therapeutic potential of VitD in RA has been evidenced by previous studies (14, 24). In addition to enhancing skeletal health in RA patients, VitD has shown the ability to reduce DAS28 by modulating the proportion of regulatory T cells (Tregs) in this population (25, 26). However, further investigations are warranted to determine the optimal dosage, treatment duration, and identify the specific patient subgroups that would derive the greatest benefits from such interventions.

The primary objective of this study, utilizing a comprehensive database, is to examine the association between mortality and 25(OH)D levels within the RA population. By analyzing extensive clinical data and conducting long-term follow-up, we aim to obtain a comprehensive understanding of the impact of VitD in the RA population and provide scientific evidence for prevention and

treatment strategies. Additionally, exploring the epidemiology and burden of RA will contribute to a better comprehension of the disease's impact on patients and serve as a reference for public health interventions.

Methods

Study population

The data used in this study originates from the National Health and Nutrition Examination Survey (NHANES), which has been conducting comprehensive and representative surveys nationwide every two years since 1999. NHANES employs complex, stratified, multi-stage probability sampling techniques to ensure accuracy. The surveys encompass household interviews and physical examinations conducted at mobile examination centers. In this study, data from nine NHANES survey cycles spanning 2001 to 2018 were employed. The dataset encompasses various factors, such as sociodemographic characteristics, body mass index, comorbidities, and VitD information, which were amalgamated into a unified dataset. Participants without mortality data were excluded from the analysis (Supplementary Figure 1).

Statistical analyses

Descriptive statistics were used to summarize the sociodemographic characteristics of the study population. VitD status were defined based on 25(OH)D levels as deficiency (< 50 nmol/L), insufficiency (50 - 75 nmol/L), or sufficiency (> 75 nmol/L) (27, 28). Median values and interquartile ranges (IQR) were utilized to summarize continuous variables, while frequencies and percentages were used to present categorical variables. Statistical tests appropriate for each variable type were utilized to assess differences in various characteristics. Specifically, chi-square tests were employed for categorical variables, and Kruskal-Wallis tests were used for continuous variables.

Cox proportional hazards regression models were employed to investigate the association between 25(OH)D levels and both all-cause mortality and cause-specific mortality. VitD status was included as a categorical variable in the model. Additionally, to investigate the association between all-cause mortality and 25(OH)D levels, subgroup analyses were performed, focusing on specific subgroups for a more targeted examination, such as age and gender. In these analyses, 25(OH)D levels was included as a continuous variable (per 10 nmol/L). To compare mortality rates among various subgroups, hazard ratios (HR) and corresponding 95% confidence intervals (CI) were computed.

To explore possible nonlinear relationships between 25(OH)D levels and mortality, restricted cubic splines (RCS) with Cox regression models were utilized. We have tested knots between 3 and 7 and selected the model with the lowest Akaike information criterion value for the RCS analysis. Adjustments were made for a range of covariates, including sex, age, race, annual household income, marital status, education level, body mass index (BMI), diabetes, hypertension, weak/failing kidneys, and total cholesterol. The statistical analyses were conducted utilizing R version 4.1.0.

Results

Study participants

After excluding 7,102 participants without VitD data, 24,207 participants without mortality data, and 45,188 participants who were not diagnosed with RA, a total of 2,290 participants were included in the study. Table 1 presents the weighted baseline characteristics of the participants categorized by status (unweighted results are presented in Supplementary Table 1). Compared to deceased individuals with RA, individuals with RA who were still alive had higher levels of 25(OH)D. The baseline characteristics of individuals with RA, categorized by their VitD status, are presented in Supplementary Tables 2 and 3, showcasing both unweighted and weighted results. Non-Hispanic White individuals had better VitD status compared to other racial groups. The married/cohabiting group had better VitD status compared to the other two groups. Annual household income and education levels were positively correlated with 25(OH)D levels (Supplementary Figure 2). BMI was negatively correlated with 25(OH)D levels (Supplementary Figure 2).

Cox proportional hazards regression

Over a follow-up period of 227 months (with a median of 91 months), a total of 586 deaths (weighted: 1,818,297) were identified. Figure 1 displays the association between 25(OH)D levels, covariates, and all-cause mortality. A robust negative correlation was observed between VitD and all-cause mortality, with a predicted HR of 0.91 (weighted: 0.87 to 0.96) per 10 nmol/L increase in 25(OH)D levels. Supplementary Figure 3 presents the unweighted results. Regarding cause-specific mortalities, the HR per 10 nmol/L increase in 25(OH)D levels was 0.88 (weighted: 0.82 to 0.95) for diseases of heart, 0.86 (weighted: 0.79 to 0.94) for malignant neoplasms and 0.92 (weighted: 0.85 to 0.99) for all other causes. However, no significant correlation was found between 25(OH)D levels and cause-specific mortalities from chronic lower respiratory diseases, influenza and pneumonia, accidents, cerebrovascular diseases, diabetes, Alzheimer's disease, nephritis, nephrotic syndrome and nephrosis diseases (Table 2). Supplementary Table 4 provides the association between VitD status and all-cause mortality. Compared to the VitD deficiency group, the HR for the VitD insufficiency group was 0.64 (weighted: 0.50 to 0.83), and for the VitD sufficiency group, it was 0.60 (weighted: 0.44 to 0.80).

Furthermore, we conducted subgroup analyses of the association between 25(OH)D levels and all-cause mortality based on selected features, including age and sex (Table 3). The HR for per 10 nmol/L increase in 25(OH)D levels was 0.92 (weighted, 0.85 to 0.99) for males and 0.91 (weighted, 0.86 to 0.98) for females. There was no significant correlation between 25(OH)D levels and

TABLE 1 Demographic characteristics of individuals with RA according to status, weighted.

Characteristic	Assumed alive (N=6362191)	Assumed deceased (N=1818297)	p-value
Sex = female (%)	3792480 (59.6)	1011732 (55.6)	0.125
Age	55.00 (45.00, 64.00)	71.00 (60.00, 79.00)	**<0.001**
Race (%)			<0.001
Mexican American	478848 (7.5)	69616 (3.8)	
Other Hispanic	363797 (5.7)	49917 (2.7)	
Non-Hispanic White	4043359 (63.6)	1391776 (76.5)	
Non-Hispanic Black	1077391 (16.9)	257166 (14.1)	
Other Race	398794 (6.3)	49819 (2.7)	
25(OH)D (nmol/L)	66.50 (50.13, 84.55)	60.60 (41.51, 77.21)	**<0.001**
Months of follow-up	101.06 (51.00, 157.00)	72.00 (34.00, 119.00)	**<0.001**
Annual household income (%)			<0.001
Under $44,999	3411020 (53.6)	1373444 (75.5)	
$45,000 to $74,999	1769551 (27.8)	383318 (21.1)	
$75,000 and over	1181620 (18.6)	61534 (3.4)	
Marital status (%)			<0.001
Married/cohabiting	4064740 (63.9)	847536 (46.6)	
Widowed/ divorced/separated	1781102 (28.0)	881905 (48.5)	
Never married	516348 (8.1)	88855 (4.9)	
Education level (%)			**<0.001**
Under high school	1368188 (21.5)	668718 (36.8)	
High school or equivalent	1718629 (27.0)	530548 (29.2)	
Above high school	3275373 (51.5)	619030 (34.0)	
BMI	29.80 (25.27, 34.80)	28.48 (24.51, 32.64)	**<0.001**
Diabetes (%)			**0.001**
No	5078261 (79.8)	1263883 (69.5)	
Borderline	180937 (2.8)	71952 (4.0)	
Yes	1102993 (17.3)	482462 (26.5)	
Hypertension = Yes (%)	3120571 (49.0)	1281196 (70.5)	**<0.001**
Weak/failing kidneys = Yes (%)	414799 (6.5)	142652 (7.8)	0.373
Total Cholesterol (mmol/L)	5.04 (4.40, 5.74)	4.92 (4.19, 5.82)	0.423

25(OH)D, 25-hydroxyvitamin D; BMI, Body mass index. The bold values means statistical significance.

mortality among individuals aged 20-59 years. However, among individuals aged 60 years and above, the HR for per 10 nmol/L increase in 25(OH)D levels was 0.86 (weighted, 0.82 to 0.90).

RCS with cox regression

To model the association between 25(OH)D levels and all-cause mortality, we applied RCS to capture the relationship in a flexible and visual manner. Specifically, we used RCS with 4 knots positioned at the 5th, 35th, 65th, and 95th centiles. Figure 2A illustrates the J-shaped pattern observed in the relationship between 25(OH)D levels and all-cause mortality. Notably, within the 25 (OH)D levels range of 59.95 to 68.2 nmol/L, there was a significant decrease in risk, with the lowest risk observed at approximately 63.6 nmol/L (unweighted). Weighted results are presented in Figure 2B, illustrating a notable increase in mortality risk for 25(OH)D levels below 59.95 nmol/L, while mortality rates consistently remained below 1.0 for levels above 59.95 nmol/L, indicating a decrease in risk.

Discussion

The observed association between serum 25(OH)D levels and mortality in individuals with RA aligns with prior research. We identified a negative correlation between serum 25(OH)D levels and all-cause mortality, with the VitD sufficiency group (> 75 nmol/L) showing the most pronounced negative correlation. These findings imply that individuals with RA may require higher levels of 25(OH) D to optimize their health status. Previous investigations have also established a negative correlation between 25(OH)D levels and mortality rates associated with CVD, cancer, and respiratory system diseases (17–20). Notably, a study conducted in Germany involving 5899 participants aged 50-75 years identified the most robust association with mortality related to respiratory system diseases (20). Moreover, a recent meta-analysis has indicated that decreased serum 25(OH)D levels are not only linked to elevated overall cardiovascular events and cardiovascular mortality but also to an increased risk of heart failure (29). Another meta-analysis demonstrated that although VitD supplementation did not reduce the overall incidence of cancer, it significantly lowered overall cancer mortality (30). Additionally, a prospective cohort study involving 493 patients in the United States showed that pancreatic cancer patients with sufficient 25(OH)D levels before diagnosis experienced an extended overall survival period (31).

Our findings both align with and differ from a recent study by Cai et al. (2023) on VitD and mortality in RA patients (32). While both studies found an inverse association between 25(OH)D levels and all-cause mortality, we identified a higher threshold for mortality risk reduction. This discrepancy may be due to our larger sample size, extended study period, and different variable selection in our models. Our additional analyses of cause-specific mortality and age stratification provide further insights, particularly

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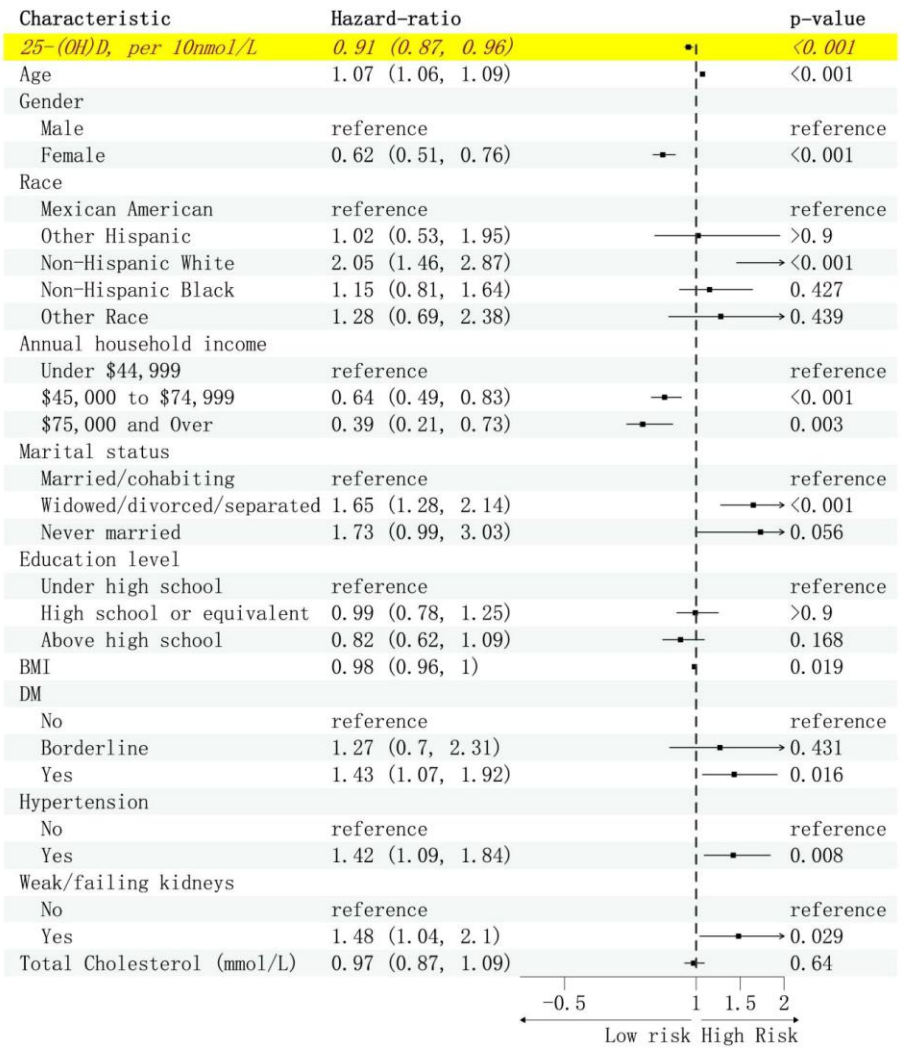

FIGURE 1
Hazard ratios (95% CI) of 25(OH)D and all covariates, weighted. The model was adjusted for age, sex, race, annual household income, marital status, education level, BMI, diabetes, hypertension, weak/failing kidneys, and total cholesterol.

TABLE 2 According to 25(OH)D levels, the HR (95% CI) of all-cause mortality among individuals with RA.

Characteristic	Unweighted		Weighted	
Cause specific mortality	HR per 10nmol/L (95% CI)	p-value	HR per 10nmol/L (95% CI)	p-value
Diseases of heart	0.88 (0.82, 0.95)	**<0.001**	0.87 (0.79, 0.97)	**0.012**
Malignant neoplasms	0.86 (0.79, 0.94)	**<0.001**	0.86 (0.76, 0.97)	**0.011**
Chronic lower respiratory diseases	1.10 (0.97, 1.24)	0.142	1.05 (0.89, 1.24)	0.546
Accidents	1.04 (0.70, 1.53)	0.846	1.08 (0.66, 1.76)	0.766
Cerebrovascular diseases	0.95 (0.81, 1.12)	0.568	0.94 (0.79, 1.12)	0.486
Alzheimer's disease	1.11 (0.91, 1.36)	0.308	1.15 (0.89, 1.50)	0.286
Diabetes mellitus	0.93 (0.74, 1.17)	0.545	0.95 (0.67, 1.35)	0.772
Influenza and pneumonia	1.07 (0.83, 1.38)	0.579	1.08 (0.88, 1.33)	0.446
Nephritis, nephrotic syndrome and nephrosis	0.84 (0.63, 1.11)	0.224	0.88 (0.70, 1.11)	0.273
All other causes	0.94 (0.87, 1.00)	0.051	0.92 (0.85, 0.99)	**0.026**

HR, Hazard ratio; CI, Confidence interval; 25(OH)D, 25-hydroxyvitamin D. The bold values means statistical significance. HRs were adjusted for age, sex, race, annual household income, marital status, education level, BMI, diabetes, hypertension, weak/failing kidneys, and total cholesterol.

TABLE 3 According to 25(OH)D levels, the HR (95% CI) of all-cause mortality in different sex and age intervals.

Characteristic	Unweighted		Weighted	
	HR per 10nmol/L (95% CI)	p-value	HR per 10nmol/L (95% CI)	p-value
Sex				
Male	0.93 (0.88, 0.98)	**0.009**	0.92 (0.85, 0.99)	**0.034**
Female	0.91 (0.86, 0.96)	**<0.001**	0.91 (0.86, 0.98)	**<0.001**
Age				
20-39	1.58 (0.68, 3.66)	0.285	3.50 (0.12, 99.30)	0.464
40-59	0.92 (0.83, 1.03)	0.15	0.93 (0.81, 1.07)	0.324
60 and over	0.92 (0.88, 0.96)	**<0.001**	0.92 (0.86, 0.97)	**<0.001**

HR, Hazard ratio; CI, Confidence interval; 25(OH)D, 25-hydroxyvitamin D. The bold values means statistical significance. HRs were adjusted for age, sex, race, annual household income, marital status, education level, BMI, diabetes, hypertension, weak/failing kidneys, and total cholesterol.

for patients aged 60 and above. These differences underscore the need for further research to establish optimal VitD levels for RA patients.

The findings of our study emphasize the distinctions observed between individuals with RA and other populations. Specifically, among individuals with RA, a robust inverse correlation is evident between serum 25(OH)D levels and all-cause mortality, particularly mortality attributed to cardiovascular diseases and malignant neoplasms. However, no significant association is observed between 25(OH)D levels and specific causes of death such as chronic lower respiratory diseases, accidents, cerebrovascular diseases, diabetes, Alzheimer's disease, nephritis, nephrotic syndrome, and nephrosis. Furthermore, there is no significant correlation between 25(OH)D levels and all-cause mortality among individuals aged 20-59 years. Nevertheless, for individuals with RA aged 60 years and above, higher levels of 25 (OH)D exhibit a link to reduced all-cause mortality, underscoring the significance of VitD supplementation in this specific population. While our study did not find a significant correlation between 25(OH)D levels and all-cause mortality among individuals aged 20 to 59 years, this does not negate the importance of maintaining adequate VitD levels in this age group. VitD plays crucial roles in bone health, immune function, and other physiological processes throughout life (27). However, the long-term effects of VitD supplementation in younger adults on mortality outcomes in later life remain unclear. To our knowledge, no longitudinal studies have directly addressed whether VitD supplementation in people aged 20 to 59 can prevent all-cause mortality when they reach 60 and above. This represents an important area for future research. Nonlinear analysis reveals an L-shaped relationship between 25(OH)D levels and all-cause mortality. The risk of mortality significantly increases for 25(OH)D levels below 59.95 nmol/L, while the HR for levels above 59.95 nmol/L consistently remains below 1. In summary, our findings highlight individuals aged 60 and above with RA as a crucial target group for VitD supplementation, with a minimum threshold of 59.95 nmol/L for 25(OH)D levels among individuals with RA.

There are multiple potential mechanisms through which the mortality rate among patients with RA can be influenced by levels of 25(OH)D. It is noteworthy that a negative correlation exists between levels of 25(OH)D and the severity of the disease in RA patients, indicating a direct contribution of lower 25(OH)D levels to mortality associated with RA (6–9). Additionally, there is an inverse association between levels of 25(OH)D and severe muscle wasting, reduced physical fitness, and decreased skeletal muscle mass in RA patients, thereby increasing the risk of osteoporotic fractures and mortality (10, 33). Moreover, insufficient levels of 25(OH)D are associated with an elevated risk of suicide in individuals with RA (23).

The pathogenesis of RA involves the interplay between T cells, B cells, and their interactions with pro-inflammatory cytokines (34). T helper (Th)-1 and Th17 cells induce pro-inflammatory cytokines such as interferon–γ, tumor necrosis factor-α, interleukin (IL)-2, IL-17, and IL-22, leading to the destruction of cartilage and erosion of bones (35, 36). Conversely, Th2 cells and cytokines have a suppressive effect on the immune progression of RA. Th2 cells mainly secrete IL-4, IL-5, IL-10, and IL-13, with IL-4 and IL-13 capable of alleviating synovial inflammation, inhibiting osteoclastogenesis, and improving bone erosion (37–39). In addition to the Th1/Th2/Th17 imbalance, RA is characterized by reduced Treg activity, including a decrease in Treg cell numbers and an imbalance between Treg and Th17 cells (40–42). The imbalance between Treg and Th17 cells aggravates arthritis and bone destruction by promoting the expression of RANKL on synovial fibroblasts (43).

Apart from its impact on the musculoskeletal system, the immunomodulatory properties of VitD are its most notable characteristic. The active form of VitD [1,25(OH)2D] effectively regulates the phenotype and physiology of various cell types. It enhances the function of Th2 and Treg cells while inhibiting the function of Th1 and Th17 cells. *In vitro*, it demonstrates significant anti-inflammatory effects on $CD4^+$ and $CD8^+$ T cells and *in vivo*, it suppresses the production of cytokines associated with Th1 and Th17 cells (44). The autocrine VitD signaling can trigger the contraction of the Th1 cell response, thereby shutting down the

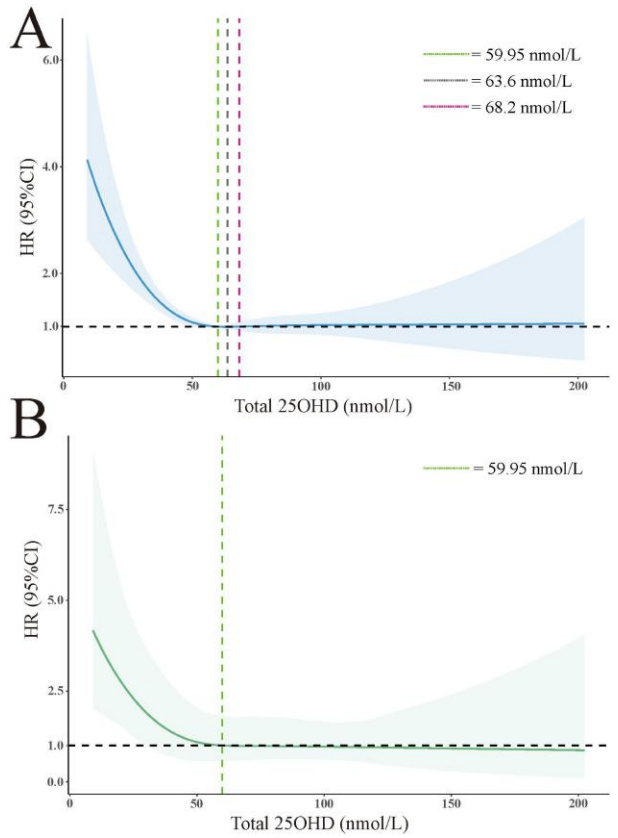

FIGURE 2
Association of total 25(OH)D level with all-cause mortality in all participants. **(A)** unweighted. **(B)** weighted. The HRs are represented by solid lines, while the 95% confidence intervals are depicted by shaded areas. Knots are positioned at the 5th, 27.5th, 50th, 72.5th, and 95th centiles of the 25(OH)D distribution. All models were adjusted for age, sex, race, annual household income, marital status, education level, BMI, diabetes, hypertension, weak/failing kidneys, and total cholesterol.

pro-inflammatory program of Th1 cells (45). Consequently, the pathogenesis of RA involves complex immune regulation, and VitD deficiency contributes to the pro-inflammatory state in RA through its impact on Th cell subsets (Th1, Th2, Treg, and Th17 cells) and their secreted cytokines.

In the context of cancer, 1,25(OH)2D exhibits inhibitory effects on the proliferation of various normal and tumor cells while promoting their terminal differentiation. This action primarily occurs through the antagonism of signaling pathways, including Wnt/β-catenin, epidermal growth factor, and transforming growth factor-β, which suppress epithelial-mesenchymal transition (46–49). Additionally, VitD can interrupt the cell cycle by directly altering cell cycle regulators that induce cell cycle arrest, thereby reducing tumor cell proliferation (50). The anticancer effects of VitD, besides inhibiting tumor cell proliferation, may also arise from controlling the growth and differentiation of the immune system (51).

VitD insufficiency is linked to various CVD, including vascular dysfunction, atherosclerosis, left ventricular hypertrophy, hypertension, and dyslipidemia (52–55). The potential mechanisms through which VitD deficiency elevates the risk of CVD are associated with the activation of VDR. VitD exerts diverse cardiovascular effects by activating VDR in cardiomyocytes and endothelial cells. It also influences the renin-angiotensin-aldosterone system, energy expenditure, adiposity, and pancreatic cell function (56). Studies in VDR knockout mice have shown that these mice display hypertension signs, including enhanced activity of the renin-angiotensin-aldosterone system, as well as cardiac hypertrophy characterized by an increased ratio of heart weight to body weight and elevated expression of natriuretic peptides (57, 58).

Our study represents the primary inquiry into the association between levels of 25(OH)D and both all-cause mortality and cause-specific mortality among individuals affected by RA. In this investigation, we have utilized the NHANES database, which incorporates a sophisticated sampling methodology and includes participants spanning a wide age range and diverse ethnic backgrounds. This comprehensive approach ensures a diverse participant pool and augments the external validity of our findings. To mitigate the impact of confounding variables, we have considered various covariates that may potentially influence serum 25(OH)D levels. By doing so, we have minimized the influence of confounding factors and gained confidence in the reliability of our statistical outcomes.

However, it is important to acknowledge the limitations of our study. A primary limitation is the reliance on self-reported data for RA classification in the NHANES dataset. Participants who reported being told by a health professional that they had RA were classified in the RA group. This method may have led to misclassification, potentially including other forms of arthritis or related conditions. The lack of clinical verification of RA diagnoses could impact the accuracy of our findings. Additionally, the NHANES dataset lacks detailed clinical information specific to RA, such as DAS28 index or number of swollen joints. Another significant limitation is the lack of data on VitD intake, both from dietary sources and supplements. The NHANES dataset, while comprehensive in many aspects, does not provide specific information on dietary VitD consumption or VitD supplementation. This limitation restricts our ability to provide a comprehensive understanding of the factors influencing VitD status in RA patients and may impact the interpretation of our results. The sample size of individuals with RA might be limited, potentially restricting our ability to conduct comprehensive analyses. Despite NHANES's sophisticated sampling design, selection bias cannot be entirely ruled out. These limitations should be considered when interpreting the study results. Future studies would benefit from more rigorous clinical classification of RA, inclusion of RA-specific clinical data, and detailed data on both dietary and supplemental VitD intake to address these gaps and provide a more complete picture of VitD status in RA.

In summary, we have observed a robust inverse association between levels of 25(OH)D and both all-cause mortality and cause-specific mortality in individuals diagnosed with RA. Specifically, this negative correlation between 25(OH)D levels and all-cause

mortality was evident only among individuals aged 60 or older with RA. Notably, a noteworthy increase in HR was identified when 25 (OH)D levels fell below 59.95 nmol/L, while mortality rates consistently remained below 1 above this threshold, indicating a decrease in risk. Based on these significant findings, we propose the adoption of routine screening for 25(OH)D levels within the RA-affected population, incorporating age stratification, particularly emphasizing individuals aged 60 or older, to facilitate appropriate VitD supplementation. Furthermore, we suggest considering a minimum threshold of 60 nmol/L for 25(OH)D levels among individuals with RA.

Data availability statement

The data used in this study are publicly available from the NHANES, conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). These data can be accessed through the CDC's NHANES website: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

Ethics statement

This study utilized publicly available, de-identified data from the NHANES, which was collected by the NCHS of the CDC. As the data used were publicly available and de-identified, the Ethics Committee of the affiliated Huaian No.1 People's Hospital of Nanjing Medical University has confirmed that no ethical approval is required.

Author contributions

YF: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. PZ: Investigation, Writing – original draft, Methodology, Conceptualization. DY: Formal analysis, Writing – original draft, Methodology. XW: Writing – original draft, Methodology. CC: Formal analysis, Writing – original draft, Methodology. ZZ: Writing – original draft, Formal analysis. YT: Methodology, Writing – original draft. JW: Writing – original draft, Methodology. SL: Writing – original draft, Methodology. JL: Writing – review & editing, Validation, Supervision, Resources. DM: Funding acquisition, Writing – review & editing, Supervision. KW: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Relationship between serum vitamin D levels and the atherogenic index of plasma: a study based on NHANES database 2011–2018

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Objective: This study aims to investigate the relationship between serum vitamin D levels and the atherogenic index of plasma (AIP) in individuals aged 20 years and above, as well as analyze potential influencing factors.

Methods: A total of 9,637 participants aged 20 years and above from the National Health and Nutrition Examination Survey (NHANES) conducted between 2011 and 2018 were included in this study. The AIP was calculated using the formula log[triglycerides (TG)/high-density lipoprotein cholesterol (HDL-C)]. Due to the skewed distribution of serum vitamin D levels in the study population, a normal transformation was performed. Weighted multivariate linear regression models were used to assess the linear relationship between the transformed serum vitamin D levels and AIP. Subgroup analysis was conducted by stratifying the data based on age, gender, and race to evaluate the stability of the relationship between serum vitamin D levels and AIP in different populations. In addition, a smooth curve fitting and generalized linear models were employed to examine the nonlinear relationship between serum vitamin D levels and AIP.

Results: After controlling for confounding factors, the multivariate linear regression analysis revealed a negative correlation between serum vitamin D levels and AIP [$\beta = -0.0065$, 95% CI: (−0.0106, −0.0024)]. This negative correlation was significant in male participants [$\beta = -0.0077$, 95% CI: (−0.0142, −0.0011)], Non-Hispanic Black participants [$\beta = -0.0135$, 95% CI: (−0.0211, −0.0059)], as well as participants aged 40–50 [$\beta = -0.0124$, 95% CI: (−0.0226, −0.0022)] and 60–70 [$\beta = -0.0118$, 95% CI: (−0.0214, −0.0023)]. Furthermore, a nonlinear relationship and saturation effect were observed between the transformed serum vitamin D levels and AIP, with a turning point at 8.5617 nmol/L.

Conclusion: Our study revealed a significant negative correlation and saturation effect between serum vitamin D levels and AIP.

KEYWORDS

vitamin D, arteriosclerosis index of plasma, NHANES, cardiovascular disease, saturation effect

Introduction

Cardiovascular disease (CVD) remains a leading cause of morbidity and mortality worldwide (1). Atherosclerosis, characterized by the accumulation of arterial wall plaques, is a major contributor to the development of cardiovascular disease (2). Its progression is influenced by various factors, including abnormalities in lipid metabolism, chronic inflammation, and vitamin D levels (3, 4). Vitamin D is primarily obtained through sunlight exposure and diet (5). As a fat-soluble vitamin, vitamin D not only plays a role in regulating calcium and phosphate metabolism but also exhibits anti-inflammatory, immune-regulatory, and antioxidant effects, making it a multifunctional hormone with pleiotropic effects. Previous studies have shown that vitamin D deficiency may impact the occurrence and progression of atherosclerosis (6). Increasing circulating levels of 25-hydroxyvitamin D(25[OH]D), the major circulating form of vitamin D, has been found to effectively reduce the risk of hypertension, stroke, and myocardial infarction (7, 8). Dyslipidemia refers to an abnormal lipid/lipoprotein profile characterized by elevated total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) levels, along with decreased high-density lipoprotein cholesterol (HDL-C) levels, which is recognized as a significant risk factor for atherosclerosis and cardiovascular disease (9).

The atherogenic index of plasma (AIP) is a novel indicator calculated as the logarithm of the ratio of triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C). It reflects the particle size and esterification rate of low-density lipoprotein cholesterol (LDL-C), which are related to lipoprotein lipase activity. Therefore, AIP is considered an important marker composed of TG and HDL-C, widely used for quantifying lipid levels and considered the optimal indicator for evaluating dyslipidemia and cardiovascular disease (CVD) (10, 11). Some studies have found AIP to be a significant and independent predictor of increased CVD risk, superior to traditional lipid parameters, and a potential biomarker for assessing the severity of coronary artery disease (12, 13). However, there is limited and conflicting research on the relationship between serum vitamin D levels and AIP. Some studies have reported a negative correlation between serum vitamin D levels and AIP, indicating that lower vitamin D levels are associated with higher AIP values (14). On the other hand, a study by Wang et al. (15) found a negative correlation between serum vitamin D concentrations and AIP in males but not in females. AIP values were higher in males with vitamin D deficiency compared to those with sufficient vitamin D levels. In order to investigate the relationship between vitamin D and AIP more accurately, we conducted this study.

The objective of this study was to elucidate the relationship between serum vitamin D levels and AIP in individuals aged 20 years and older, and further explore the influencing factors of this relationship. By analyzing a large sample dataset from NHANES 2011–2018, we aim to provide more reliable evidence to support the role of vitamin D in the prevention and treatment of atherosclerosis.

Materials and methods

Study population

The NHANES database is a population-based nationwide survey that provides information on population nutrition and health. The NHANES database can be publicly accessed at www.cdc.gov/nchs/nhanes. Our study utilized NHANES data from 2011 to 2018. Among the 39,156 participants, there were 16,539 individuals below the age of 20, 2,124 with missing serum vitamin D data, and 10,856 with missing TG or HDL data. After applying these exclusion criteria, a total of 9,637 participants were included in the clinical analysis (Figure 1).

Study variables

The independent variable in this study is serum Vitamin D. Due to the skewed distribution of serum Vitamin D in the study population (Figure 2A), a normal transformation was applied to serum Vitamin D in the article (Figure 2B). The dependent variable is the calculated plasma atherogenic index (AIP), which is computed using the formula AIP = log (TG [mg/dL]/HDL-C [mg/dL]). The following variables were included as covariates in the clinical analysis: age, gender, race, ratio of family income to poverty (PIR), body mass index (BMI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine (Scr), TC, alkaline phosphatase (ALP), Calcium (Ca), and Phosphorus (P). The examination section related to clinical and laboratory evaluations was provided by well-trained medical experts. Detailed procedures and measurement methods for each variable can be found at www.cdc.gov/nchs/nhanes.

Statistical analysis

All analyses were conducted using the weights from the NHANES examination sample, and the baseline characteristics of all participants included in the final analysis were described as mean ± standard deviation (continuous variables) or percentage (categorical variables). A weighted multivariable linear regression model was used to assess the linear relationship between serum Vitamin D after normal transformation and AIP, and subgroup analyses were performed to evaluate the linear relationship between serum Vitamin D and AIP in different populations by stratifying for age, gender, and race. Additionally, a smoothed curve fitting and generalized linear models were employed to investigate the non-linear relationship between serum Vitamin D and AIP. The inflection point (if it existed) was calculated using a two-segment linear regression model with a recursive algorithm. A p value <0.05 was considered statistically significant. We utilized EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA) and the statistical software package R (http://www.Rproject.org, The R Foundation) for the modeling process. Frequency distribution plots for serum Vitamin D and serum Vitamin D after normal transformation were generated using Origin (version: 2024).

Results

Baseline characteristics of participants

After applying the inclusion and exclusion criteria, a total of 9,637 participants met the criteria. The population characteristics, weighted

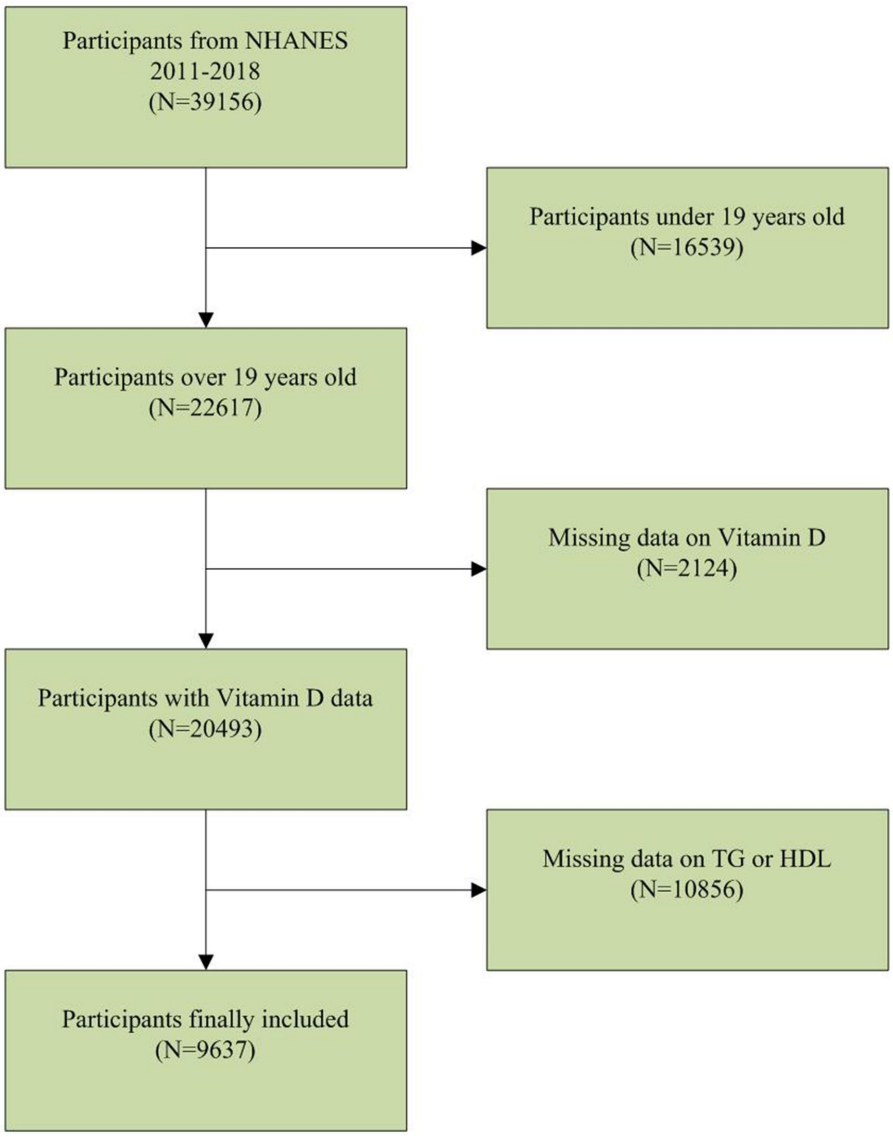

FIGURE 1
Baseline characteristics of participants.

according to the quartiles of serum Vitamin D after normal transformation (Q1: 6.65 ± 0.82 nmol/L; Q2: 8.31 ± 0.34 nmol/L; Q3: 9.40 ± 0.32 nmol/L; Q4: 10.99 ± 0.93 nmol/L), are presented in Table 1. Significant differences were observed in age, gender, race, PIR, BMI, ALT, BUN, Scr, TC, TG, HDL, LDL, ALP, and Ca among different groups based on serum Vitamin D quartiles (Q1–Q4). Compared to the lowest quartile, individuals in the highest quartile were more likely to be older, female, have a higher PIR, a higher proportion of Non-Hispanic Whites, and higher levels of BUN, Scr, TC, HDL, LDL, and Ca. Conversely, they exhibited lower BMI, ALT, TG, and ALP levels (Table 1).

Association between serum vitamin D and AIP

Table 2 presents the relationship between serum Vitamin D and Atherogenic Index of Plasma (AIP). Three weighted multivariate linear regression models were constructed. In the unadjusted model, there was a negative correlation between serum Vitamin D and AIP [$\beta = -0.0169$, 95% CI: (−0.0209, −0.0129)]. After controlling for confounding factors, this negative correlation persisted in Model 2 [$\beta = -0.0258$, 95% CI: (−0.0301, −0.0215)] and Model 3 [$\beta = -0.0065$, 95% CI: (−0.0106, −0.0024)]. When serum Vitamin D was converted from a continuous variable to a categorical variable (quartiles), individuals in the highest quartile had an AIP that was 0.0266 lower than those in the lowest quartile.

Subgroup analysis

Subgroup analyses were performed in this study to assess the stability of the relationship between Atherogenic Index of Plasma (AIP) and serum Vitamin D across different population backgrounds. The results showed a significant negative correlation

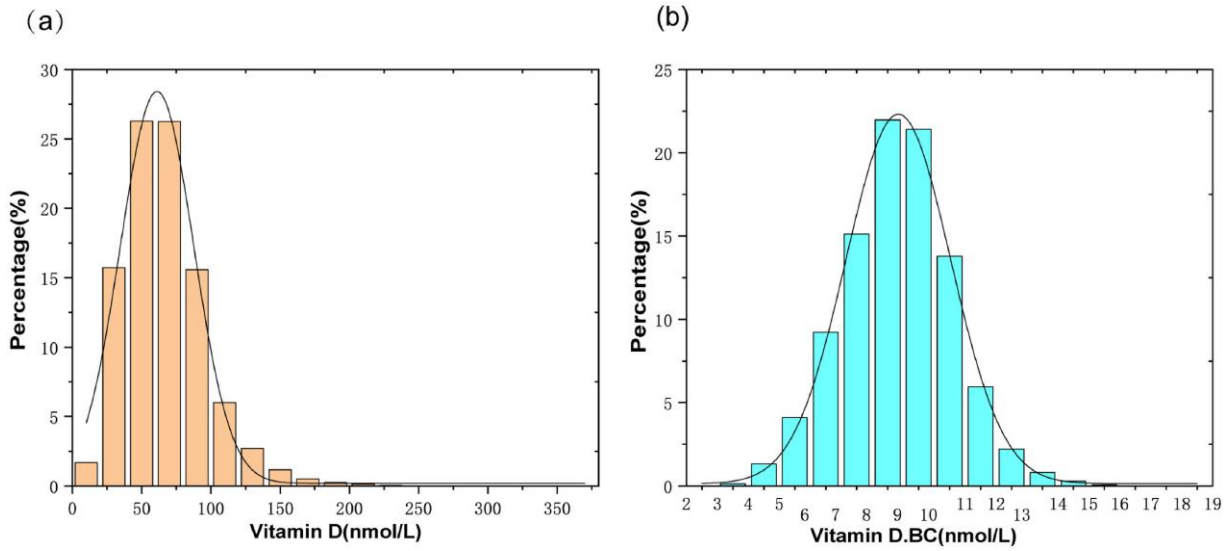

FIGURE 2
(A) Histogram of serum vitamin D distribution; **(B)** Histogram of serum vitamin D distribution after normalization transformation(Vitamin D.BC).

between serum Vitamin D and AIP among male participants [−0.0077 (−0.0142, −0.0011)]. When stratified by race, Non-Hispanic Black participants exhibited a significant negative correlation between serum Vitamin D and AIP [−0.0135 (−0.0211, −0.0059)]. Among different age groups, participants aged 40–50 years [−0.0124 (−0.0226, −0.0022)] and 60–70 years [−0.0118 (−0.0214, −0.0023)] demonstrated a significant negative correlation between serum Vitamin D and AIP. Other factors did not significantly influence the relationship between serum Vitamin D and AIP (Table 3).

Non-linearity and saturation effect analysis between serum vitamin D and AIP

A smoothed curve fitting was used to describe the non-linear association and saturation phenomenon between serum Vitamin D and Atherogenic Index of Plasma (AIP) (Figure 3). The results showed that the saturation point for the relationship between serum Vitamin D (after undergoing a normal transformation) and AIP in all participants was 8.5617 nmol/L. When the transformed serum Vitamin D was below 8.5617 nmol/L, the effect size was 0.0130; whereas when the transformed serum Vitamin D exceeded 8.5617 nmol/L, the effect size changed to −0.0184 (Table 4).

Discussion

In our study, we found a negative correlation between serum vitamin D levels and AIP. Further subgroup analysis revealed significant associations between serum vitamin D levels and AIP in male participants, non-Hispanic black individuals, and those aged between 40–50 and 60–70 years. Interestingly, we observed an inverted L-shaped relationship between logarithmically transformed serum vitamin D levels and AIP, with a turning point at 8.5617 nmol/L.

Cardiovascular disease (CVD) poses a significant threat to human health, with high global incidence, mortality, and disability rates (16). AIP has been identified as one of the strongest biomarkers for predicting CVD risk (17). AIP reflects the balance between pro-atherogenic lipids such as triglycerides and anti-atherogenic lipids like high-density lipoprotein cholesterol (18). It can serve as an adjunct to individual lipid profiles. AIP is a better determinant of HDL-C particle fractionation than conventional lipid parameters (19, 20). Studies have shown that higher AIP values are associated with an increased risk of coronary artery disease (CAD) (21). In recent years, an increasing number of studies have demonstrated an association between low 25(OH)D levels and increased cardiovascular disease risk and all-cause mortality (22, 23). Ge et al. (24) found in their study among rural Chinese population that serum 25(OH)D3 concentration was correlated with lipid levels, with varying associations between individuals with normal lipid levels and those with abnormal lipid levels; as serum 25(OH)D3 levels increased, the incidence of lipid abnormalities decreased. A cross-sectional study conducted among middle-aged and elderly Chinese population found a positive correlation between vitamin D deficiency and abnormal lipid profiles and AIP (25). Our study also revealed similar findings in individuals aged 20 and above.

In this study, we found a significant correlation between serum vitamin D levels and AIP. After adjusting for confounding factors, we observed a significant negative correlation between serum vitamin D levels and AIP in males. However, in females, our results showed a p value greater than 0.05, indicating no significant relationship between serum vitamin D levels and AIP after adjusting for confounding factors. This finding is consistent with some previous studies. Wang et al. (15) reported a negative correlation between serum 25(OH)D concentration and AIP in males but not in females. Furthermore, males with vitamin D deficiency had higher AIP values compared to males with sufficient vitamin D. Naganuma et al. (26) reported that low serum 25(OH)D levels were associated with increased atherosclerosis risk in adolescent boys but not in girls. Several factors may contribute to these gender-specific differences.

TABLE 1 Weighted characteristics of 9,637 participants included in this study.

Characteristics	Vitamin D(nmol/L)				
	Q1	Q2	Q3	Q4	*p* value
	N = 2,405	*N* = 2,410	*N* = 2,399	*N* = 2,423	
Age	42.66 ± 15.78	43.93 ± 16.24	47.68 ± 16.47	55.21 ± 16.22	<0.0001
Sex (%)					<0.0001
Male	48.06	56.12	53.86	39.55	
Female	51.94	43.88	46.14	60.45	
Race (%)					<0.0001
Mexican American	16.71	13.14	6.64	2.34	
Other Hispanic	6.13	9.33	7.87	3.19	
Non-Hispanic White	37.98	56.80	71.16	83.81	
Non-Hispanic Black	28.00	10.00	6.05	4.20	
Other race	11.19	10.73	8.28	6.46	
PIR	2.35 ± 1.57	2.72 ± 1.63	3.08 ± 1.64	3.35 ± 1.60	<0.0001
BMI (kg/m^2)	31.23 ± 8.37	29.86 ± 7.08	29.02 ± 6.56	27.90 ± 6.19	<0.0001
ALT (U/L)	25.47 ± 18.66	26.40 ± 19.71	24.15 ± 15.89	23.39 ± 14.72	<0.0001
AST (U/L)	25.04 ± 17.08	24.63 ± 22.40	24.08 ± 13.49	24.88 ± 13.54	0.2111
BUN (mg/dL)	4.40 ± 2.07	4.75 ± 1.75	5.05 ± 1.72	5.36 ± 2.09	<0.0001
Scr (mg/dL)	0.85 ± 0.57	0.86 ± 0.37	0.88 ± 0.29	0.91 ± 0.39	<0.0001
TC (mg/dL)	186.76 ± 41.20	186.85 ± 41.00	191.84 ± 40.34	194.76 ± 41.47	<0.0001
TG (mg/dL)	121.76 ± 136.28	125.27 ± 110.17	120.50 ± 84.83	115.10 ± 84.36	0.0035
HDL (mg/dL)	51.68 ± 15.08	50.53 ± 14.18	53.47 ± 15.39	59.78 ± 18.71	<0.0001
LDL (mg/dL)	111.48 ± 34.85	111.72 ± 35.56	114.51 ± 34.37	112.47 ± 36.03	0.0143
ALP (U/L)	72.80 ± 25.83	69.72 ± 29.69	67.64 ± 22.84	66.58 ± 21.53	<0.0001
Ca (mg/dL)	9.28 ± 0.34	9.29 ± 0.33	9.34 ± 0.33	9.37 ± 0.37	<0.0001
P (mg/dL)	3.65 ± 0.60	3.63 ± 0.53	3.64 ± 0.54	3.66 ± 0.54	0.2298
Vitamin D (nmol/L)	34.50 ± 8.04	54.75 ± 4.95	72.12 ± 5.56	105.26 ± 23.43	<0.0001
Vitamin D.BC	6.65 ± 0.82	8.31 ± 0.34	9.40 ± 0.32	10.99 ± 0.93	<0.0001
AIP	0.29 ± 0.35	0.32 ± 0.34	0.29 ± 0.34	0.23 ± 0.33	<0.0001

Mean ± SD for continuous variables: the *p* value was calculated by the weighted linear regression model. (%), for categorical variables: the *p* value was calculated by the weighted chi-square test. Q, Quartile; PIR, Ratio of family income to poverty; BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood urea nitrogen; SCr, Serum creatinine; TC, Total cholesterol; TG, Triglyceride; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; ALP, Alkaline phosphatase; ALP, Alkaline phosphatase; Vitamin D, 25OHD2 + 25OHD3; Vitamin D.BC, Vitamin D after normalization transformation; AIP, Atherogenic index of plasma.

First, it could be related to hormonal changes. Hormones have profound effects on lipid metabolism (27), which could have different impacts on the relationship between vitamin D and AIP in males and females. Sex hormones play an important role in the regulation of lipid metabolism. A sex-stratified meta-analysis identified lipid-related loci showing sex-biased effects on both autosomes and the X chromosome, with associations with the pleiotropy of sex hormones, highlighting the important role of sex hormone regulation in lipid metabolism (28). These hormones may interact with the vitamin D signaling pathway, leading to gender-specific effects on AIP. Secondly, vitamin D signaling has multiple effects outside the skeletal system, including regulation of cell proliferation, immune and muscle function, skin differentiation and reproduction, as well as vascular and metabolic properties (29). These effects may manifest differently in males and females, resulting in differential associations between serum vitamin D levels and AIP in both sexes. Lastly, there are differences in dietary patterns, exercise habits, sunlight exposure, and other factors between males and females, which may influence the synthesis and absorption of vitamin D. Therefore, these behavioral and lifestyle differences between male and female populations may modulate the relationship between serum vitamin D levels and AIP.

We conducted a stratified analysis based on race and found a significant negative correlation between serum vitamin D and AIP among non-Hispanic Black individuals, while no such phenomenon was observed in other races. This may be related to genetic variations that affect vitamin D metabolism. In a cross-sectional study of multi-ethnic populations with atherosclerosis (MESA), significant racial differences were found in vitamin D metabolism indicators. Compared to Black participants, White participants had significantly higher concentrations of 25-hydroxyvitamin D in their serum. The ratios of circulating vitamin D metabolites indicated lower *CYP27B1* activity and higher *CYP24A1* activity among White participants. Differences

TABLE 2 Association between VitaminD.BC (nmol/L) and AIP.

Exposure	Model 1, [β (95% CI)]	Model 2, [β (95% CI)]	Model 3, [β (95% CI)]
VitaminD.BC (continuous)	−0.0169 (−0.0209,−0.0129)	−0.0258 (−0.0301,−0.0215)	−0.0065 (−0.0106,−0.0024)
Vitamin D.BC (quartile)			
Quartile 1	Reference	Reference	Reference
Quartile 2	0.0274 (0.0064, 0.0485)	−0.0153 (−0.0360, 0.0054)	0.0063 (−0.0127, 0.0252)
Quartile 3	−0.0028 (−0.0232, 0.0176)	−0.0536 (−0.0742, −0.0330)	0.0043 (−0.0147, 0.0234)
Quartile 4	−0.0627 (−0.0824, −0.0429)	−0.1098 (−0.1307, −0.0889)	−0.0266 (−0.0462, −0.0070)
p for trend	<0.001	<0.001	0.002

Model 1: no covariates were adjusted. Model 2: age and gender were adjusted. Model 3: age, gender, race, PIR, BMI, ALT, AST, BUN, Scr, TC, LDL, ALP, Ca, and P were adjusted.

TABLE 3 Association between Vitamin D.BC and AIP stratified by sex, race and age.

	Model 1, β (95% CI) *p* value	Model 2, β (95% CI) *p* value	Model 3, β (95% CI) *p* value
Stratified by gender			
Male	−0.0149 (−0.0214, −0.0084)***	−0.0275 (−0.0347, −0.0203)***	−0.0077 (−0.0142, −0.0011)*
Female	−0.0112 (−0.0159, −0.0064)***	−0.0249 (−0.0302, −0.0196)***	−0.0024 (−0.0075, 0.0028)
Stratified by race			
Mexican American	−0.0019 (−0.0146, 0.0107)	−0.0101 (−0.0229, 0.0026)	0.0020 (−0.0095, 0.0136)
Other Hispanic	−0.0094 (−0.0250, 0.0062)	−0.0120 (−0.0273, 0.0033)	0.0086 (−0.0057, 0.0229)
Non-Hispanic White	−0.0330 (−0.0400, −0.0259)***	−0.0311 (−0.0383, −0.0240)***	−0.0063 (−0.0130, 0.0004)
Non-Hispanic Black	−0.0183 (−0.0256, −0.0110)***	−0.0249 (−0.0325, −0.0174)***	−0.0135 (−0.0211, −0.0059)***
Other Race	−0.0082 (−0.0180, 0.0015)	−0.0138 (−0.0241, −0.0036)**	−0.0050 (−0.0149, 0.0049)
Stratified by age			
Aged<30	−0.0142 (−0.0249, −0.0035)**	−0.0220 (−0.0336, −0.0104)***	−0.0066 (−0.0170, 0.0038)
30 ≤ aged<40	−0.0149 (−0.0258, −0.0041)**	−0.0190 (−0.0306, −0.0074)**	−0.0047 (−0.0152, 0.0058)
40 ≤ aged<50	−0.0246 (−0.0354, −0.0138)***	−0.0313 (−0.0426, −0.0201)***	−0.0124 (−0.0226, −0.0022)**
50 ≤ aged<60	−0.0281 (−0.0381, −0.0181)***	−0.0302 (−0.0404, −0.0199)***	−0.0069 (−0.0171, 0.0033)
60 ≤ aged<70	−0.0312 (−0.0401, −0.0224)***	−0.0311 (−0.0401, −0.0221)***	−0.0118 (−0.0214, −0.0023)*
70 ≤ aged<80	−0.0142 (−0.0233, −0.0051)**	−0.0148 (−0.0239, −0.0057)*	−0.0016 (−0.0105, 0.0072)

In subgroup analyses stratified by gender, race, age, Model 1: no covariates were adjusted. Model 2: age and sex were adjusted. Model 3: age, gender, race, PIR, BMI, ALT, AST, BUN, Scr, TC, LDL, ALP, Ca, and P were adjusted.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. $p < 0.05$ was considered statistically significant.

in vitamin D-binding globulin haplotypes were also observed (30). These genetic variations may lead to different ways of metabolizing and utilizing vitamin D among non-Hispanic Black participants compared to other races. Therefore, the relationship between vitamin D and AIP may exhibit different patterns.

When stratified by age, we found a significant negative correlation between serum vitamin D levels and plasma atherogenic index of plasma (AIP) among participants aged 40–50 and 60–70. With increasing age, there are various changes in the metabolism and activity of vitamin D. The ability of the skin to produce vitamin D3 decreases with age, reducing by 13% every decade (31). The resistance of the intestines to 1,25-dihydroxyvitamin D increases, affecting calcium absorption in the gut. Among various organs involved in calcium metabolism, the number of vitamin D receptors decreases with age, and the activity of 1α-hydroxylase decreases mainly due to declining kidney function, leading to reduced activation of vitamin D (32). Vitamin D deficiency is common in the elderly population as a result. Age-related factors also include changes in hormone and bone morphogenetic protein levels. In conclusion, this significant negative correlation may be attributed to age-related changes in vitamin D metabolism, cumulative effects of vitamin D deficiency, alterations in lipid metabolism, and complex interactions with other age-related factors (33). Further research is needed to understand the exact mechanisms and clinical significance of this age-specific relationship.

In our study, we employed a smooth curve fitting to describe the non-linear association and saturation phenomenon between serum vitamin D and AIP. The saturation effect value of 8.5617 nmol/L may have physiological significance in the relationship between vitamin D and AIP. When the serum vitamin D level, transformed into a normal distribution, is below this threshold, its regulatory effect on AIP is limited, while beyond this threshold, vitamin D may exert a stronger negative regulatory effect. The interpretation of these findings may also need to consider the metabolism and mechanisms of action of vitamin D. Vitamin D mediates its biological effects in cells by binding to vitamin D receptors. The saturation phenomenon may reflect the saturation or regulatory mechanism of these receptors, resulting in a non-linear relationship and the manifestation of a saturation effect for vitamin D. Similar studies have found a U-shaped association between

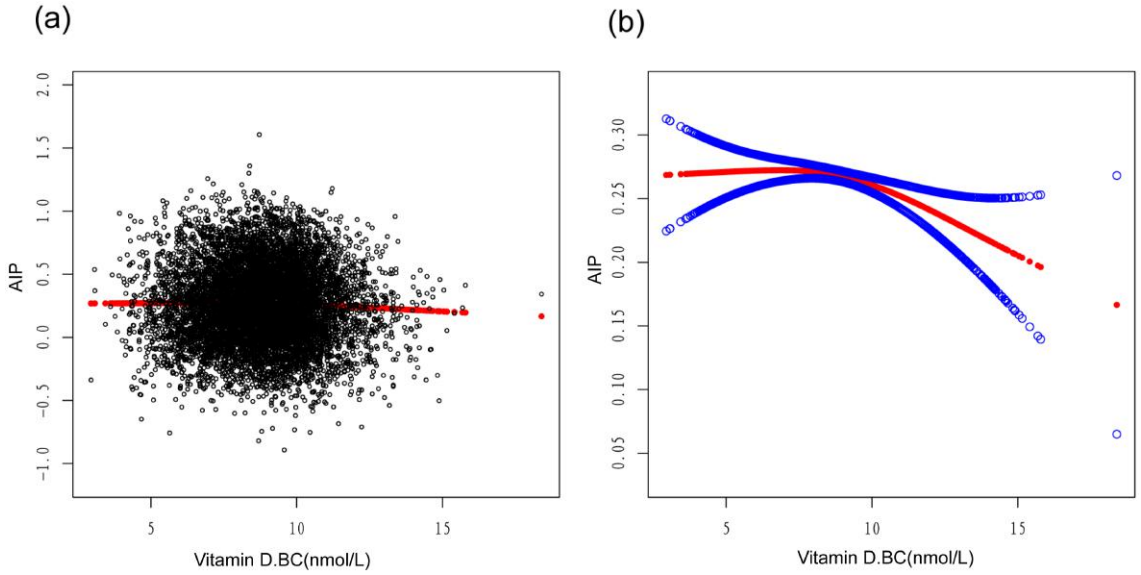

FIGURE 3
Association between vitamin D.BC and AIP (The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% confidence interval from the fit).

TABLE 4 Saturation effect analysis of SerumVitaminD.BC (nmol/L) on AIP.

AIP	Model: saturation effect analysis [β (95% CI) *p* value]
SerumVitaminD.BC turning point (K)	8.5617
<K, effect1	0.0130 (0.0044, 0.0217) 0.0032
>K, effect2	−0.0184 (−0.0245, −0.0122) <0.0001
Log-likelihood ratio	<0.001

Age, gender, race, PIR, BMI, ALT, AST, BUN, Scr, TC, LDL, ALP, Ca, and P were adjusted.

serum 25(OH)D levels and CVD risk, suggesting a non-linear relationship between vitamin D and CVD prevalence (34). It should be noted that although we observed the saturation effect between serum vitamin D and AIP, further research is still needed to determine the optimal level of vitamin D. An animal model experiment showed that high-dose vitamin D, as an adjunct to simvastatin therapy, was superior to omega-3 levels in improving TG, HDL, and AIP (35). Additionally, maintaining an appropriate serum level of vitamin D appears to be crucial for calcium homeostasis and cardiovascular risk, blood pressure regulation, stroke incidence, metabolic syndrome, and peripheral arterial disease. Vitamin D exerts beneficial effects on the cardiovascular system by reducing the activity of the renin-angiotensin-aldosterone system (RAAS), lowering blood pressure, and possessing anti-inflammatory, anti-proliferative, anti-hypertensive, anti-fibrotic, anti-diabetic, and anti-thrombotic properties (36). These potential benefits further underscore the significance of determining the optimal level of vitamin D and suggest that vitamin D may play a vital role in the prevention and treatment of cardiovascular diseases.

The investigation of the non-linear association between vitamin D and AIP may contribute to a better understanding of the biological effects of vitamin D and its impact on cardiovascular health. One of the major strengths of this study was the utilization of the NHANES database, which provided a large representative sample of the general population. By employing rigorous statistical analysis and adjusting for confounding factors, we were able to establish a strong association between serum vitamin D levels and AIP. However, there were several limitations to our study. Firstly, the cross-sectional design of NHANES limited our ability to establish causality. Secondly, reliance on self-reported data may have introduced recall bias. Thirdly, our findings may not be generalizable to populations beyond the NHANES sample. Future prospective studies and clinical trials are necessary to confirm our findings and explore underlying mechanisms.

Conclusion

In conclusion, our study uncovered a negative correlation between serum vitamin D levels and AIP, suggesting a potential protective role against atherosclerosis and cardiovascular diseases. Subgroup analyses stratified by gender, race, and age revealed interesting variations in the associations. These findings highlight the significance of optimizing vitamin D status as a prospective preventive strategy for cardiovascular diseases, including atherosclerosis. Further research, including prospective studies and clinical trials, is warranted to validate our findings and elucidate the underlying mechanisms.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: www.cdc.gov/nchs/nhanes.

Ethics statement

The studies involving humans were approved by Board of the National Center for Health Statistics. The studies were conducted in

accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from Publicly available datasets were analyzed in this study. This data can be found here: www.cdc.gov/nchs/nhanes. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

TH: Data curation, Formal analysis, Methodology, Writing – original draft. YZ: Conceptualization, Methodology, Writing – review & editing. ZC: Formal analysis, Writing – review & editing. JS: Formal analysis, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The effect of vitamin D supplementation on antibiotic use: a meta-analysis based on randomized controlled trials

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Objective: This meta-analysis explores the impact of vitamin D supplementation on antibiotic utilization.

Methods: We systematically searched for relevant randomized controlled trials (RCTs) in PubMed, Web of Science, EMBASE, and Science Direct from inception to April 2024. These trials compared antibiotic use rates between groups receiving vitamin D supplements and placebo.

Results: We included seven RCTs involving 35,160 participants. There was no significant difference in antibiotic use between the two groups in the general population (Odds Ratio [OR] = 0.98, $p = 0.232$), including elderly participants (OR = 0.98, $p = 0.295$). However, antibiotic use was lower in the intervention group compared to the placebo group among participants under 70 years of age (OR = 0.95, $p = 0.015$), those with relative vitamin D deficiency [25(OH)D < 75 nmol/L, OR = 0.95, $p = 0.024$; 25(OH)D < 50 nmol/L, OR = 0.96, $p = 0.026$], and those with respiratory tract infections (RTIs) (OR = 0.51, 95% CI: 0.24–1.08, $p = 0.080$), although these differences were not statistically significant for RTIs.

Conclusion: Vitamin D supplementation does not affect antibiotic use in the general population. However, it does reduce antibiotic utilization in individuals with RTIs, relative vitamin D deficiency, or aged below 70 years.

Systematic review registration: This meta-analysis adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, and is registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42024543246.

KEYWORDS

vitamin D supplementation, antibiotic use, infection, respiratory tract infections, vitamin D deficiency, meta-analysis

Introduction

A spatial modeling study published in 2021 reported that global antibiotic consumption increased by 46% from 2000 to 2018, peaking at 40.1 defined daily doses (DDDs) in 2018 (1). Antibiotics, pivotal in reducing the morbidity and mortality associated with many infectious diseases, are considered life-saving drugs. However, their widespread availability and perceived

cost-effectiveness have led to increased irrational and misuse (2). This is compounded by a lack of adequate awareness among both the public and medical professionals. Such overuse has accelerated the development of drug-resistant bacteria, posing a significant threat to global health due to the ensuing antibiotic resistance (3).

In response to the critical issue of antibiotic resistance, no effective alternatives have been developed, which necessitates the continuous development of new antibiotics (4). Recent studies suggest that combining antibiotics with non-antibiotic compounds could improve treatment outcomes against multi-resistant bacteria by possibly aiding in antibacterial action or repairing metabolic defects (5). For instance, it has been demonstrated that the addition of substrates like glucose or alanine can enhance the tricarboxylic acid cycle, thereby increasing bacterial uptake of antibiotics and improving their efficacy (6). Additionally, existing studies have suggested that vitamin D deficiency also played an important impact on extra-skeletal diseases, especially on respiratory tract infections (RTIs) such as bacterial pneumonia and acute respiratory infections (ARIs) (7). Notably, vitamin D has been recognized for its substantial immunomodulatory effects, such as activating immune cell chemotaxis, enhancing phagocytic capabilities of macrophages (8), and inducing the production of antimicrobial peptides (9). These properties suggest that vitamin D could serve as a supportive antimicrobial agent. A prior meta-analysis involving 25 randomized controlled trials (RCTs) indicated that vitamin D supplementation could lower the incidence of ARIs (10). Moreover, a prospective observational study in Sweden showed that vitamin D supplementation was associated with a reduction in antibiotic usage days (11). There was, in addition, a cohort study shown that low serum vitamin D levels were an independent predictor of adverse outcomes of COVID-19 and might result in higher levels of inflammation and more serious tissue damage in patients with severe or non-severe cases (12). Despite these findings, there were no meta-analyses that explore the effect of vitamin D supplementation on antibiotic use. In light of these considerations, this study aims to review published RCTs to perform a meta-analysis assessing the relationship between vitamin D supplementation and antibiotic usage frequency in adults.

Methods

Search strategy

This meta-analysis adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (13), and is registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42024543246. We conducted a systematic search for RCTs examining the effects of vitamin D supplementation on antibiotic use from inception to April 2024. Searches were performed using PubMed, Web of Science, EMBASE, and Science Direct, with keywords including (vitamin D) AND (antibiosis OR antibiotic OR antibiotics OR anti-infection OR infection). A secondary search was conducted through the references of all identified studies to ensure the comprehensiveness of our search.

Selection and exclusion criteria

The inclusion and exclusion of studies were guided by the PICOS (participants, intervention, comparison, outcomes, and study design) framework (14). The inclusion criteria were as follows: (1) participants: adults aged 16 years or older, or those at high risk of antibiotic use due to certain diseases (excluding tuberculosis); (2) intervention: oral administration of vitamin D in the intervention group; (3) comparison: placebo given to the control group; (4) outcomes: measures related to antibiotic use; (5) study Design: only RCTs were considered.

Exclusion criteria included: (1) studies where relevant data could not be extracted or were unsuitable for statistical analysis; (2) studies where the full text was unavailable; (3) studies not published in English; (4) studies with outdated or superseded publications, where articles with the most recent and comprehensive data were given preference.

Data extraction and quality assessment

Data extraction was performed independently by two investigators using a predefined form. This form captured essential information including the authors, year of publication, country, clinical trial number, participant characteristics (age, number, recruitment year, physical condition, and vitamin D supplementation regimen), and antibiotic-related outcomes.

Risk of bias was assessed according to the guidelines provided by the Cochrane Collaboration Network (15). The assessment covered several domains: (1) random sequence generation (to address selection bias), (2) allocation concealment (to address selection bias), (3) blinding of participants and personnel (to mitigate performance bias), (4) blinding of outcome assessment (to mitigate detection bias), (5) completeness of outcome data (to address attrition bias), (6) selective reporting (to address reporting bias), and (7) other potential biases. Each domain was rated as 'high risk,' 'low risk,' or 'unclear risk'. Disagreements between investigators were resolved through discussion to reach a consensus.

Statistical analysis

Statistical analyses were conducted using Stata Software version 12.0 (Stata Corporation LLC, College Station, United States). The impact of vitamin D supplementation on antibiotic use was evaluated using odds ratios (ORs) and 95% confidence intervals (CIs). Heterogeneity among the studies was assessed with a chi-square test and quantified using the I^2 statistic. I^2 values over 50% were considered indicative of significant heterogeneity, values between 25 and 50% indicated moderate heterogeneity, and values below 25% indicated low heterogeneity (16).

Due to potential variations among study participants and differences in study protocols, analyses were performed using a

Abbreviations: DDDs, Defined Daily Doses; RTIs, Respiratory Tract Infections; ARIs, Acute Respiratory Infections; RCTs, Randomized Controlled Trials; OR, Odds Ratio; CI: Confidence Interval; COPD, Chronic Obstructive Pulmonary Disease; VDR, Vitamin D Receptor; PRRs, Pattern Recognition Receptors; IL, Interleukin; CAMP, Cathelicidin Antimicrobial Peptide; Defb2, Human β2-Defensins; NO, Nitric Oxide; mTOR, Mammalian Target of Rapamycin; ROS, Reactive Oxygen Species; No, Number.

random-effects model to enhance the reliability of the results. Subgroup analyses were conducted to explore the sources of heterogeneity further. Publication bias was assessed using Begg's test, and sensitivity analyses were performed to verify the stability of the findings. All statistical tests were two-sided, with a significance threshold set at $p < 0.05$.

Results

Study selection

From four electronic databases, a search identified 55,352 records under the specified research strategy. No additional records were identified through other sources. After removing duplicates, 18,740 records remained. Screening of titles and abstracts led to the exclusion of 18,715 records due to low relevance, leaving 25 full-text articles for detailed evaluation. Out of these, 18 articles were excluded for the following reasons: 5 were non-RCTs, 1 had insufficient data, and 12 did not report antibiotic-related outcomes. Ultimately, 7 studies met the inclusion criteria and were included in the meta-analysis. The detailed retrieval process is illustrated in Figure 1.

Characteristics and quality assessment of included studies

The 7 RCTs (17–23), spanning from 2007 to 2022 and cited as references, investigated the relationship between vitamin D supplementation and antibiotic use, involving 35,160 participants from five countries (Sweden, United Kingdom, Australia, Netherlands, New Zealand). The intervention groups in these studies received oral vitamin D supplementation, while control groups were administered a placebo. Six (17, 18, 20–23) of the studies involved oral cholecalciferol and one (19) involved oral D-Peals capsules produced in Denmark. Duration of intervention varied: three studies (17, 20, 23) had durations exceeding 1 year, and the remaining four (18, 19, 21, 22) were conducted for 1 year or less.

Participants' physical conditions varied across studies: one (18) involved participants with antibody deficiencies or frequent RTIs; one (19) included individuals with 25(OH)D levels below 75 nmol/L; one (21) focused on patients with a history of chronic obstructive pulmonary disease (COPD) exacerbation within the last 12 months and 25(OH)D levels below 50 nmol/L; one (17) targeted patients with low trauma and osteoporotic fractures; and three (20, 22, 23) included elderly individuals

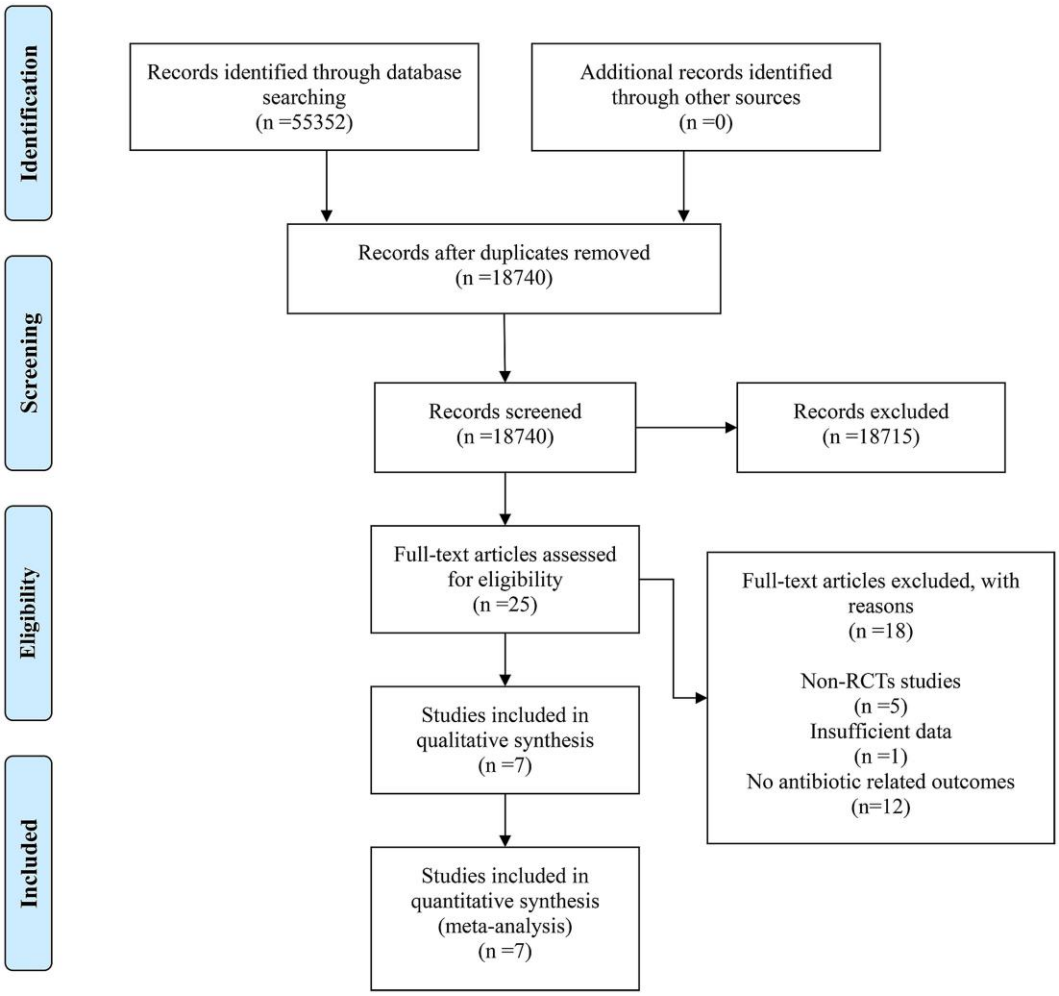

FIGURE 1
Flow chart of study selection.

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TABLE 1 Characteristics of all the studies included in the meta-analysis.

Author	Year	Country	No. of participants		Participant characteristics		Dosage and duration of Vitamin D
			Vitamin D	Placebo	Age (year)	Physical condition	
			Male/Female				
Bergman, Peter	2012	Sweden	18/52	20/50	18–75	Antibody deficiency or frequent RTIs	4,000 IU daily for 1 year
Jolliffe, David A.	2022	UK	498/1052	1040/2060	≥16	25(OH)D < 75 nmol/L	800 IU daily for 6 months
			506/1044				3,200 IU daily for 6 months
Pham, Hai	2022	Australia	5336/4426	5327/4408	60–84	/	60,000 IU monthly for 5 years
Rafiq, R.	2022	Netherlands	46/28	55/26	≥40	A COPD exacerbation in the last 12 months before screening, 25(OH)D < 50 nmol/L	16,800 IU weekly for 1 year
Tran, Bich	2014	Australia	113/97	110/95	60–84	/	30,000 IU monthly for ≤12 months
			109/96				60,000 IU monthly for ≤12 months
Wu, Zhenqiang	2021	New Zealand	1512/1046	1457/1093	50–84	/	100,000 IU monthly for a median of 3.3 years
Avenell, A.	2007	UK	1737	1703	≥ 70	Low trauma, osteoporotic fracture	800 IU daily for 18 months

RTIs, respiratory tract infections; No, number; COPD, chronic obstructive pulmonary disease.

from the general community. Further characteristics and details of the included studies are presented in Table 1 and Supplementary Table S1.

The risk of bias was assessed for each study using the Cochrane Collaboration's tool, as depicted in Supplementary Figures S1, S2. One study (19) was deemed to have a high risk of selection and performance bias due to inadequate concealment of treatment allocation and lack of stratified randomization. Another study (21) was identified as having a high risk of bias due to not achieving the designed sample size. Three studies (18, 22, 23) presented challenges in determining other biases. Overall, the studies were considered to be of high quality (Table 2).

Analysis of the primary result

Pooling the results from seven RCTs (17–23), no significant difference in antibiotic use was observed between the intervention group receiving vitamin D supplementation and the placebo group (OR = 0.98, 95% CI: 0.94–1.02, $p = 0.232$, Figure 2). However, there was moderate heterogeneity among the studies ($I^2 = 40.9\%$).

Subgroup analysis

Subgroup analyses were conducted based on participant age thresholds. For participants aged ≥70 years, no statistical difference in antibiotic use was observed between the intervention and control groups (OR = 0.99, $p = 0.731$). Conversely, among participants aged <70 years, the intervention group exhibited a reduced use of antibiotics compared to the control group (OR = 0.95, $p = 0.015$). Additional subgroup analysis among older adults similarly showed no significant differences in antibiotic use between the groups (OR = 0.98, $p = 0.295$).

Vitamin D concentration levels of less than 75 nmol/L or 50 nmol/L were considered relatively inadequate (24). Among participants with 25(OH)D levels <75 nmol/L, four RCTs (19–21, 23) indicated reduced antibiotic use in the intervention group compared to the control group (OR = 0.95, $p = 0.024$). For participants with 25(OH)D levels <50 nmol/L, results from three RCTs (17, 18, 22) demonstrated that the vitamin D-receiving group used fewer antibiotics than the placebo group (OR = 0.96, $p = 0.026$), with no significant heterogeneity ($I^2 = 0\%$).

Regarding vitamin D dosage, participants were categorized based on daily intake exceeding 2000 IU (high-dose supplementation group) or not (low-dose supplementation group). Neither the high-dose group (OR = 0.95, $p = 0.765$) nor the low-dose group (OR = 0.88, $p = 0.111$) showed significant differences in antibiotic use. Similarly, based on the duration of supplementation, no significant differences were found either for durations greater than 1 year (OR = 0.99, $p = 0.109$) or less than or equal to 1 year (OR = 0.77, $p = 0.205$).

For participants suffering from RTIs in two RCTs (18, 19) the intervention group exhibited a lower rate of antibiotic utilization

TABLE 2 Subgroup analysis of the effect of vitamin D supplementation on antibiotic use.

Subgroup	No. of studies	OR (95% CI)	*P*	I^2
Age				
Older adults	4	0.98 [0.96, 1.01]	0.295	31.8%
≥70	3	0.99 [0.96, 1.03]	0.731	23.7%
<70	2	0.95 [0.91, 0.99]	0.015	0.0%
25(OH)D concentration				
<75 nmol/L	4	0.95 [0.92, 0.99]	0.024	0.0%
<50 nmol/L	3	0.96 [0.92, 0.99]	0.026	0.0%
Doses of vitamin D				
> 2000 IU/day	4	0.95 [0.66, 1.36]	0.765	49.4%
≤2000 IU/day	4	0.88 [0.75, 1.03]	0.111	39.0%
Supplementation time of vitamin D				
>1 years	3	0.99 [0.97, 1.00]	0.109	0.0%
≤1 year	4	0.77 [0.52, 1.15]	0.205	38.7%
Infection type				
RTIs	2	0.51 [0.24, 1.08]	0.080	22.4%

RTIs, respiratory tract infections; OR, odds ratio; CI, confidence interval; No, number.

compared to the control group, though the difference was not statistically significant (OR = 0.51, 95% CI: 0.24–1.08, $p = 0.080$).

Publication bias and sensitivity analysis

Publication bias was assessed using Begg's test, which indicated no significant bias in the results related to the effect of vitamin D on antibiotic use ($p = 0.230$, Supplementary Figure S3). Sensitivity analysis was conducted by sequentially excluding each study, confirming that the results remained stable (Supplementary Figure S4).

Discussion

In this meta-analysis of seven RCTs (17–23), no significant association was observed between vitamin D supplementation and the risk of antibiotic use, encompassing elderly participants and various subgroup analyses concerning dosage and duration of vitamin D supplementation. However, among participants under 70 years of age, those with relative vitamin D deficiency, or those suffering from RTIs, vitamin D supplementation appears to reduce antibiotic usage.

Vitamin D, recognized as a multifunctional health-promoting molecule (25), is absorbed into the bloodstream and converted in the kidneys to its active form, 1,25(OH)2D3, via the catalytic action of 1α-hydroxylase (26). It primarily exerts its effects through interaction with the vitamin D receptor (VDR), facilitating the receptor complex's migration to the nucleus and modulating the expression of numerous genes related to immune regulation and infection control (27). The immunomodulatory mechanisms of vitamin D include enhancing phagocytosis and chemotaxis of innate immune cells such as macrophages and monocytes, thereby improving pathogen clearance (28); inducing dendritic cell tolerance through the expression of CYP27B1, which enhances localized concentrations of active vitamin D at infection sites (29); and integrating with pattern recognition receptors (PRRs) to detect microbial signals and activate downstream infection-fighting pathways (30). Additionally, vitamin D helps regulate inflammatory responses by inhibiting pro-inflammatory cytokines like interleukin-2 (IL-2) and promoting the production of anti-inflammatory cytokines such as IL-10 (31).

Beyond immune regulation, vitamin D also plays a significant role in anti-infection processes: it promotes the production of host defense peptides, including cathelicidin antimicrobial peptide (CAMP) and human β2-defensins (Defb2) (32), with vitamin D response elements directly influencing their gene expression (33). It mediates the synthesis of nitric oxide (NO), which enhances antimicrobial activity (34) and may reduce the viability of *Streptococcus pneumoniae* and the emergence of antibiotic resistance (35). Furthermore, vitamin D reduces the activity of mammalian target of rapamycin (mTOR), supports the recruitment of ATG16L1 by NOD2 to induce autophagy, and aids in the elimination of intracellular bacteria (36). The antioxidant properties of vitamin D help eliminate harmful reactive oxygen species (ROS), moderating inflammation and maintaining mitochondrial function, which is crucial in reducing TNF-α-induced lung epithelial inflammation and mitochondrial autophagy (37, 38). In conclusion, while vitamin D's role in reducing antibiotic use was not uniformly observed across all study participants, its various immunomodulatory and antibacterial properties theoretically support the reduction of antibiotic usage, particularly in certain subpopulations.

Although the mechanisms discussed were not substantiated across all participants in our study, several factors could explain these findings. Firstly, certain disease states and environmental factors might suppress antimicrobial peptide levels, thus diminishing the anti-infective efficacy of vitamin D. Studies have demonstrated that diabetes can down-regulate the expression of antimicrobial peptides (39), and prolonged exposure to polluted air reduces these peptide levels in mice (40). Secondly, genetic variations in the VDR genes affect individual responsiveness to vitamin D. A meta-analysis has shown that genotypes with the TaqI polymorphism and the FF variant in the FokI gene are more responsive to vitamin D supplementation (41). Thirdly, in individuals who are not deficient in vitamin D, additional supplementation may lead to inefficient binding of the vitamin to its receptors, as excess vitamin D is converted to 1,24,25(OH)2D3, which has minimal affinity for VDR (42). This hypothesis is supported by subgroup analysis indicating that high-dose vitamin D supplementation does not significantly reduce the risk of antibiotic use. This is consistent with a single-center RCT finding that additional vitamin D supplementation did not decrease hospital-acquired infection rates among sepsis patients (43), suggesting that vitamin D supplementation may not universally contribute to reduced antibiotic use.

Moreover, while one study suggested that high doses of vitamin D could decrease inflammation levels and enhance anti-infection capabilities (44), our subgroup analysis found no significant benefits from either low or high doses of vitamin D supplementation in reducing antibiotic use. Possible explanations include: (1) active vitamin D maintains a dynamic equilibrium in the body, with excess being converted to an inactive form that cannot be effectively utilized (42); (2) variability in the effectiveness of different vitamin D supplementation regimens; and (3) the prevalence of adequate serum vitamin D levels among our study participants, which could obscure any potential benefits for those with insufficient vitamin D levels.

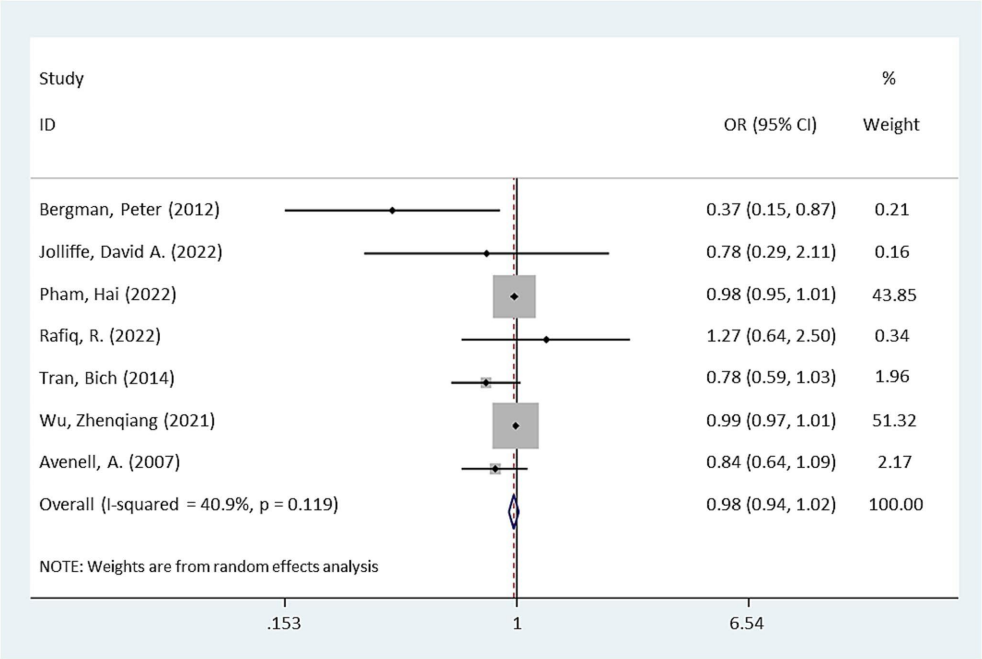

FIGURE 2
Forest plot of the effect of vitamin D supplementation on antibiotic use ($p = 0.232$).

Previous studies have demonstrated that vitamin D supplementation had a more phenomenal impact on participants with vitamin D deficiency (45).

Emerging evidence underscores the association between low serum vitamin D levels and increased infection risk (46–48). The Third National Health and Nutrition Examination Survey demonstrated an inverse relationship between serum vitamin D levels and recent upper respiratory tract infections in the American population (49). Furthermore, a meta-analysis confirmed that vitamin D deficiency heightens susceptibility to serious infections (50). Our subgroup analysis for participants with low serum vitamin D levels (25(OH)D < 50 nmol/L or <75 nmol/L) corroborates these findings, suggesting that vitamin D deficiency may compromise neutralizing antibody production and immune cell function (51). Therefore, vitamin D supplementation could potentially enhance immune responses and infection resistance.

Specifically, studies indicate that antibiotic usage is more prevalent among the elderly, women (52), and individuals in poorer health (53). Despite the theoretical benefits of vitamin D in boosting immunity among the elderly to combat infections, our findings did not support this hypothesis for participants aged ≥70 years. This discrepancy may be attributable to several factors: (1) age-related decline in organ function associated with calcium metabolism may lead to decreased expression of VDR, resulting in inefficient utilization of vitamin D (54); (2) the prevalence of chronic kidney disease in older adults impairs the kidneys' ability to activate vitamin D (55); (3) parathyroid hormone, known to enhance the synthesis of 1α-hydroxylase (56)—which converts vitamin D to its active form—is often diminished in older women, as evidenced by higher rates of hypoparathyroidism in this group (57); (4) comorbid conditions such as diabetes can negatively affect antimicrobial peptide production (39). A meta-analysis involving 41,552 elderly patients revealed that vitamin D supplementation did not significantly reduce the incidence of ARIs or lower respiratory infections (58), further supporting our observations.

Conversely, vitamin D supplementation was found to be beneficial in participants under 70 years of age. Possible explanations include: (1) younger individuals often engage in higher levels of physical activity, which may enhance vitamin D metabolism in adipose tissue (59); (2) higher physiological requirements and lower dietary intake of vitamin D in younger populations may lead to more pronounced deficiencies (60, 61), which supplementation can effectively address.

As for the type of infection, many studies available now have confirmed that vitamin D can relieve the symptoms of infection or reduce the onset of RTIs. This was consistent with our findings. The reasons may be as follows: (1) vitamin D can promote the repair of epithelial cells and inhibit the apoptosis of epithelial cells, thereby improving lung function (62); (2) vitamin D can enhance mucosal immunity including respiratory mucosa (63); (3) existing study showed that daily supplementation of vitamin D could increase the antibacterial activity of airway surface fluids (37); (4) RTIs activated T and B lymphocytes and significantly up-regulated the expression of VDR (64, 65). Thus, vitamin D supplementation can conduce to the promotion of the ability to fight infection and reduce the risk of antibiotic use in people suffering from RTIs.

For all we know, this was the first meta-analysis to conduct a comprehensive and systematic exploration of the relationship between the antibiotic use and the supplementation of vitamin D. It could provide a reference value for the field of antibiotic use. Importantly, the meta-analysis was based on the RCTs with high quality. Nevertheless, there were some limitations in the present meta-analysis. Firstly, the number of studies was limited. Besides, on account of the limited data, we could not perform further subgroup

analysis including the sex or the body mass index. Moreover, certain heterogeneity was produced due to the different physical conditions of the subjects and various programs of vitamin D supplementation.

Conclusion

This meta-analysis revealed that vitamin D supplementation does not significantly impact antibiotic usage in the general population, including elderly individuals. The regimen of vitamin D supplementation also showed no effect on antibiotic use. However, vitamin D supplementation may be beneficial in reducing antibiotic use among individuals under 70 years of age, those with relative vitamin D deficiency, or those suffering from RTIs. To substantiate these findings, more multicenter RCTs on a larger scale are necessary.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MW: Writing – original draft, Conceptualization. YW: Writing – original draft, Methodology, Investigation, Data curation. ZX: Writing – original draft, Investigation. YZ: Writing – original draft, Formal analysis. TH: Writing – review & editing. BC: Writing – review & editing, Supervision, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1502835/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Risk of bias graph for quality assessment of the included RCTs.

SUPPLEMENTARY FIGURE S2
Risk of bias summary for quality assessment of the included RCTs.

SUPPLEMENTARY FIGURE S3
Begg's test for assessing publication bias of included RCTs (p=0.230).

SUPPLEMENTARY FIGURE S4
Sensitivity analysis for testing the stability of statistical results.

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The effects of vitamin D levels on physical, mental health, and sleep quality in adults: a comprehensive investigation

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Background: Vitamin D, essential hormone for endocrine, autocrine, and paracrine functions. A billion people are deficient globally which contributing to numerous health issues. This study explores the link between vitamin D levels and sleep quality, impacting mental and physical health in adults.

Methods: This prospective cross-sectional study was conducted at Nims Hospital, Jaipur, involving 484 adults' participants. Blood samples were collected for serum 25(OH) D measurements. Data were gathered using the SF-36 and ISI questionnaires to assess health and sleep quality.

Results: Higher vitamin D levels were strongly linked to better physical health, including physical function ($r = 0.642$, $p < 0.001$), general health ($r = 0.560$, $p < 0.001$), and PCS score ($r = 0.441$, $p < 0.001$). Vitamin D also positively impacted social functioning ($r = 0.096$, $p = 0.035$) and was negatively related to ISI scores ($r = -0.112$, $p = 0.014$).

Conclusion: The study highlights a strong link between higher vitamin D levels and improved physical and mental health, with significant negative correlation to ISI scores. This underscores the importance of adequate vitamin D for overall well-being. The findings call for urgent measures to address vitamin D deficiency and further research into its health impacts.

KEYWORDS

vitamin D, SF-36, ISI, mental health, physical health, sleep

1 Introduction

Vitamin D, often celebrated as the "sunshine vitamin," is a pivotal hormone with broad-reaching roles in the body's endocrine, autocrine, and paracrine systems. This essential nutrient supports bone health, enhances calcium absorption, modulates immune function, and plays a preventive role against a wide range of health conditions, including osteoporosis, autoimmune diseases, and certain cancers (1–3). Despite its

importance, about a billion people globally are affected by insufficient vitamin D levels, including 7.4% of Canadians, 5.9% of Americans, and 13% of Europeans (4–6). Deficiency rates are similarly high in countries like Australia, Turkey, and across regions in Africa and South America, indicating a pressing global concern (1, 3, 7, 8). India also reflects this trend, with studies showing deficiency rates between 50% and 94% across diverse population groups (9).

The health implications of vitamin D deficiency are profound. Beyond its foundational role in calcium and phosphorus metabolism, vitamin D insufficiency is linked to an increased risk of chronic illnesses such as osteoporosis, rickets, and serious conditions including breast and colon cancer, cardiovascular disease, hypertension, and diabetes. It has even been associated with neurodegenerative conditions such as Parkinson's disease, while also impacting mental health by contributing to mood disorders, like depression, which are exacerbated by low vitamin D levels (10). These links became especially relevant during the COVID-19 pandemic, when vitamin D's immune-modulating properties gained attention for potentially reducing complications related to the virus (11). These extensive connections highlight how vitamin D plays an essential role not only in preventing physical diseases but also in supporting mental well-being, influencing disease susceptibility and overall health across all ages (12–20). In 2019 alone, insufficient vitamin D levels were estimated to affect the mental health of 293 million individuals worldwide (21).

Vitamin D's influence extends to sleep, an essential aspect of daily functioning and well-being. A deficiency in vitamin D is connected to various sleep disorders, including restless legs syndrome and sleep apnea, which can further exacerbate chronic health conditions through disrupted sleep cycles and diminished sleep quality (22–26). Sleep itself is regulated by a complex interaction of circadian rhythms, neural pathways, and hormonal signals originating in the hypothalamus, which responds to environmental cues like light. Nevertheless, sleep disorders remain underdiagnosed, even within healthcare settings. Research underscores that both excessive sleep and sleep deprivation are associated with heightened risks of diabetes, hypertension, cancers, and increased mortality rates (27).

The relationship between vitamin D insufficiency and sleep disruption has thus emerged as a significant area of study. Vitamin D receptors play a role in producing melatonin, the hormone central to regulating the sleep–wake cycle, supporting mood, and sustaining an optimal quality of life (28–31). Furthermore, geographic and cultural factors, particularly in India, intensify the need to investigate this link. While India enjoys abundant sunlight, limited outdoor exposure, urban lifestyles, and dietary preferences restrict sufficient vitamin D synthesis among large segments of the population. Additionally, factors like dietary restrictions and skin pigmentation can further reduce vitamin D synthesis, elevating deficiency risks. Given the high prevalence of vitamin D insufficiency in India and its potential impact on both physical and mental health, a comprehensive study examining the connection between vitamin D levels and sleep quality is crucial. This investigation aims to explore how these factors jointly influence well-being in adults aged 18 and older, with insights that could improve health outcomes and highlight the importance of vitamin D in maintaining quality of life across diverse lifestyles and geographies.

2 Materials and methods

2.1 Study design and patient enrollment

A prospective cross-sectional study was conducted at the NIMS hospital, A Tertiary Teaching Care Hospital, A unit of NIMS University Rajasthan, Jaipur, North India for a duration of 8 months (from August 2023 to March 2024). Patients aged 18 years and above visiting the outpatient (OPD) and inpatient (IPD) departments of general medicine without a clinical diagnosis of any bone-related disease and history of bone fractures were enrolled. Whereas, the patients undergoing hormone replacement therapy, diagnosed with HIV, malignant conditions, chronic kidney disease, coronary artery disease, angina, myocardial infarction, history of experiencing a heart attack and drug abuse, consumption of vitamin D supplements, women who were pregnant or breastfeeding, and the people who did not willingly participate or give their consent were excluded. The blood samples of the enrolled participants were collected for serum 25(OH) D measurements in the department of biochemistry and laboratory services at the NIMS hospital. The vitamin D and serum calcium levels were determined as per the standard guidelines of Virtus 5600 (32). Checking serum 25(OH)D levels was done with the Virtus 5600 integrated system [Model number J56001308].

2.2 Study recruitment and data collection process

548 patients were screened using the planned inclusion and exclusion criteria. Of these, 64 patients did not meet the inclusion criteria, leaving 484 patients who were eligible to join the study after giving written consent. A structured data collection form with the SF-36 questionnaire, a commonly used tool for evaluating patients' mental and physical health, was used to collect the data. Physical health and mental health are the two-summary metrics derived from its eight scales. Physical functioning (10 components), role-physical (four items), bodily pain (two items), and overall health make up the physical health summary score (five items). Physical health (four items), social skills (two items), emotional and role-related skills (three items), and mental health make up the mental health measure (five items). These domains evaluate energy levels, social engagement, the impact of emotional issues on daily activities, and overall mental well-being. The RAND Healthcare SF-36 scoring instructions from the RAND Corporation are used to decide the scores. Along with the Insomnia Severity Index (ISI) questionnaire, domain scores range from 0 to 100, with higher scores indicating better health-related quality of life and lower scores indicating worse health. The seven-item ISI questionnaire was used to find out the insomnia's nature, severity, and effects. Participants were asked about sleep maintenance, sleep onset, sleep dissatisfaction, early morning waking issues, daytime functioning, and anxiety related to sleep difficulties for the past month. Four levels of insomnia severity were found based on the answers: no insomnia (0–7 level), sub-threshold insomnia (8–14 score), moderate insomnia (15–21 score), and severe insomnia (22–28 score) (33–41), encompassing socio-demographic details (such as name, age, gender, address, residential area, marital status, history of smoking, alcohol and tobacco use, milk consumption, and sun exposure), clinical presentation (concerns related to bones, history of

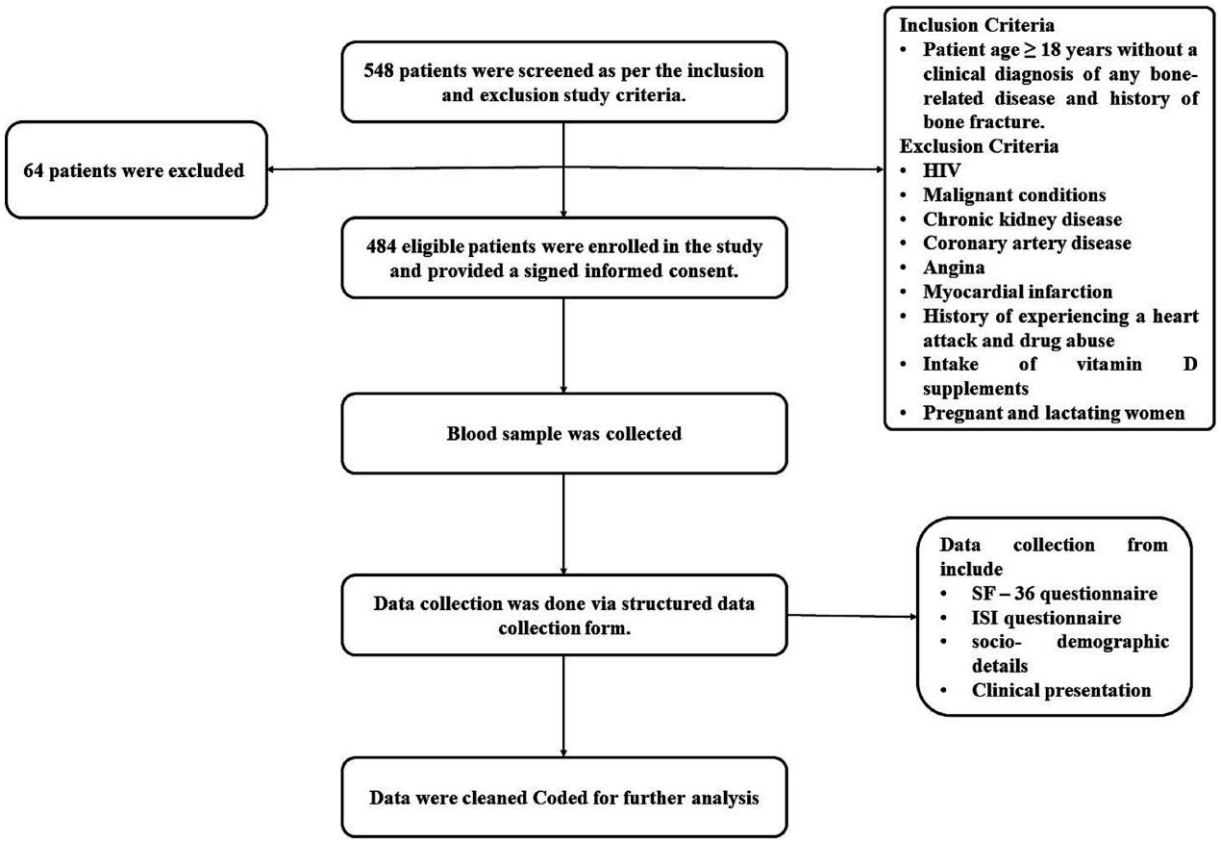

FIGURE 1
Flowchart illustrating the methodology of the study.

bone fractures, comorbidity, current symptoms, and present diagnosis), and all questions from the questionnaires were duly addressed (Figure 1).

2.3 Ethics

The research conducted in this study obtained approval from the Institutional Ethics Committee (IEC) at NIMS University Rajasthan. The reference number for this approval is NIMSUR/IEC/2023/677. It is important to note that throughout the entire research process, strict adherence was maintained to the ethical principles delineated in the Declaration of Helsinki, which was established in 1975.

2.4 Statistical analysis

The statistical analysis for this study was conducted using two software tools: Excel (version 2019) and IBM SPSS (version 28.0). Power analysis with a power of 0.80 with an alpha level of 0.05, and a confidence interval of 95% was used to reach the required sample size (42). Continuous variables were summarized by calculating the mean and standard deviation, while categorical variables were presented through median, frequency, and percentage measures. To compare categorical variables, the Fisher exact test was employed, whereas for comparing quantities, the *t*-test was utilized. This approach ensured comprehensive and accurate data interpretation in accordance with the study's objectives.

3 Results

A total of 484 participants were enrolled with an average age of 46.60 ± 16.4 years, 43.8% male and 56.2% female. The majority of participants followed a vegetarian diet (84.5%), while 12.7% followed a non-vegetarian diet. There were people from rural areas (59.3% of the participants) and people from cities (40.7%). 25.2% were smokers, with 14.3% being former smokers. Additionally, 19.6% were identified as alcoholics, with 13.4% being former alcoholics. Regarding chewing tobacco, 10.7% were consumers, while 27.1% were former consumers. The average milk consumption was 202.6 ± 133.48 mL/day, while the average sun exposure received was 16.79 ± 8.14 min/day. Based on Body Mass Index (BMI) categorization, 9.03% of participants were classified as underweight, 80.78% as normal weight, 7.64% as overweight, and 2.47% as obese (Table 1).

3.1 Laboratory investigations

There were 70.5% subject with low (≤30 ng/mL), 28.7% normal (30.1–100 ng/mL), and 0.8% high (>100 ng/mL) vitamin D levels with an average of 26.43 ± 15.41 ng/mL. 46.5% subjects were having low

TABLE 1 Demographic and anthropometric details of the subjects enrolled in the study.

Variables	*N* (%)
Total subjects (*n*)	**484**
Age (mean ± SD, years)	46.60 ± 16.4
Gender	
Male	212 (43.8%)
Female	272 (56.2%)
Dietary pattern	
Veg	60 (84.5%)
Non-Veg	9 (12.7%)
Area of population	
Rural population	287 (59.3%)
Urban population	197 (40.7%)
Smoking status	
Smoking former	69 (14.3%)
Smoking current	122 (25.2%)
Smoking never	293 (60.5%)
Alcohol status	
Alcohol former	65 (13.4%)
Alcohol current	95 (19.6%)
Alcohol never	324 (66.9%)
Tobacco status	
Tobacco former	131 (27.1%)
Tobacco current	52 (10.7%)
Tobacco never	301 (62.2%)
Body mass index (kg/m^2)	
Underweight	44 (9.03%)
Normal weight	391 (80.78%)
Overweight	37 (7.64%)
Obese	12 (2.47%)
Milk consumption (mean ± SD, mL)	202.6 ± 133.48
Sun exposure (mean ± SD, min)	16.79 ± 8.14

All the continuous variables are presented in mean ± standard deviation (SD) and categorical variables are presented in number and percentage (%).

(≤8.7 mg/dL), 51.7% normal (8.8–10.7 mg/dL), and 1.9% high (>10.8 mg/dL) serum calcium levels, accounting for an average of 8.72 ± 1.19 mg/dL (Table 2).

3.2 Scores of participants for the mental and physical components (SF-36)

For the mental component, the overall score is 27.83 ± 11.36, and it is split into four areas: Vitality, Social Functioning, Role Emotional, and Mental Health. These areas have average scores of 76.15 ± 13.94, 77.81 ± 15.79, 32.57 ± 38.94, and 51.80 ± 11.62, respectively. Physical function, Body Pain, Role Physical and General Health each have an average score of 46.94 ± 15.54, 34.75 ± 26.79, 45.17 ± 14.76, and 44.29 ± 5.17, respectively, on the physical component summary score (52.21 ± 13.83) (Table 3).

TABLE 2 Vitamin D and serum calcium levels of the individuals enrolled in the study.

Variables	N (%)
Vitamin D (ng/mL), Mean ± SD	26.43 ± 15.41
Low 1–30	341 (70.5%)
Normal 30.1–100	139 (28.7%)
High 100.1+	4 (0.8%)
Serum calcium (mg/dL), Mean ± SD	8.72 ± 1.19
Low 0–8.7, *n* (%)	225 (46.5%)
Normal 8.8–10.7	250 (51.7%)
High 10.8+	9 (1.9%)

All the continuous variables are presented in mean ± standard deviation (SD) and categorical variables are presented in number and percentage (%).

TABLE 3 Mental and physical component scores of the individuals enrolled in the study (SF-36).

Variables	Mean ± SD
PCS	52.21 ± 13.83
Physical function	46.94 ± 15.54
Role physical	34.75 ± 26.79
Body pain	45.17 ± 14.76
General health	44.29 ± 5.17
MCS	27.83 ± 11.36
Vitality	76.15 ± 13.94
Social functioning	77.81 ± 15.79
Role emotional	32.57 ± 38.94
Mental health	51.80 ± 11.62

All the continuous variables are presented in mean ± standard deviation (SD).

3.3 Insomnia severity index scores of the enrolled participants

The average ISI score of participants was 18.73 ± 6.34, with 3.9% having no clinically significant insomnia, 28.1% with subthreshold insomnia, 14.7% with moderate insomnia, and 53.3% with severe clinical insomnia (Table 4).

3.4 Correlation of vitamin D and serum calcium levels with SF-36 (physical, mental health) and ISI score

There was a strong link between higher levels of vitamin D and various aspects of physical health, including the physical function score ($r = 0.642$, $p < 0.001$), the general health score ($r = 0.560$, $p < 0.001$), and the physical component summary (PCS) score ($r = 0.441$, $p < 0.001$). Concerning mental health, there was a positive correlation between social functioning and vitamin D levels ($r = 0.096$, $p = 0.035$), while Insomnia Severity Index (ISI) scores were significantly negatively

TABLE 4 Insomnia severity index of the subjects enrolled in the study.

Variables	*N* (%)
Total (mean ± SD)	18.73 ± 6.34
No clinically significant insomnia	19 (3.9%)
Subthreshold insomnia	136 (28.1%)
Clinical insomnia (moderate severity)	71 (14.7%)
Clinical insomnia (severe)	258 (53.3%)

All the continuous variables are presented in mean ± standard deviation (SD) and categorical variables are presented in number and percentage (%).

related to vitamin D levels ($r = -0.112$, $p = 0.014$). Additionally, there was a significant positive correlation between serum calcium levels and various aspects of physical health, such as general health ($r = 0.185$, $p = 0.021$) and the PCS score ($r = 0.018$, $p = 0.047$). For mental health, role physical does not show any significant correlation with serum calcium ($r = 0.050$, $p = 0.276$). However, social functioning and role emotional also exhibited no significant correlations with serum calcium ($r = 0.006$, $p = 0.894$ and $r = -0.051$, $p = 0.267$, respectively). ISI scores had a negligible association with serum calcium ($r = 0.008$, $p < 0.001$) (Table 5; Figure 2).

3.5 Correlation between SF-36 (physical, mental health) and ISI score

There was a significant negative correlation between ISI score and various aspects of physical health, such as physical function ($r = -0.193$, $p < 0.001$) and general health ($r = -0.107$, $p = 0.018$), with the physical component summary (PCS) score showing a similar trend ($r = -0.096$, $p = 0.035$). Regarding mental health, social functioning was negatively correlated with ISI score ($r = -0.189$, $p < 0.001$) (Table 6).

4 Discussion

The primary objective of this research was to examine the correlation between vitamin D levels, health-related quality of life (HRQoL) as measured by the SF-36 questionnaire, and the severity of insomnia in adults aged 18 and older without bone-related diseases. Results revealed strong associations between vitamin D levels, several dimensions of HRQoL, and insomnia severity, indicating that vitamin D status significantly affects both physical and mental health outcomes.

Laboratory analyses showed a significant prevalence of vitamin D deficiency, with 70.5% of participants having serum levels below 30 ng/mL, consistent with prior studies emphasizing the high occurrence of vitamin D deficiency in regions with limited sunlight exposure or diets low in vitamin D-rich foods (43). This widespread insufficiency underscores a potential need for interventions to improve vitamin D status as a preventive health measure.

Vitamin D levels were positively correlated with several physical HRQoL dimensions, including physical functioning, general health perception, and overall physical component summary (PCS) scores. Higher vitamin D levels were associated with better physical functioning, fewer limitations in physical role activities, and more positive general health perceptions. This suggests a potential role of vitamin D in reducing physical health limitations and enhancing overall well-being. Additionally, a positive correlation between vitamin D levels and social functioning was observed, indicating that sufficient vitamin D may support better social engagement and interpersonal interactions. While vitamin D's impact on social functioning is indirect and mediated through its effects on mental health (e.g., depression, social withdrawal), it highlights the multifactorial nature of social behavior, shaped by biological, psychological, and environmental factors (44, 45). Therefore, the complexity of these interactions results in weak but significant correlations, reflecting the nuanced role of vitamin D in social and emotional health (31, 46).

Notably, ISI scores, reflecting insomnia severity, were negatively correlated with vitamin D levels. This suggests that adequate vitamin D levels may help mitigate the severity of insomnia, likely due to the influence of the vitamin D receptor (VDR) on genes related to hormones, neurotransmitters, and circadian rhythm regulation. VDR activation promotes the synthesis of serotonin and melatonin, which are essential for sleep initiation and maintenance. Disrupted VDR signaling can impair these processes, potentially leading to poor sleep quality and disrupted sleep patterns (47). This observation reinforces the relevance of monitoring vitamin D status as part of diagnosing and managing sleep disorders, with implications for both mental and physical health.

Additionally, serum calcium levels were positively associated with physical health aspects within SF-36, particularly in general health and PCS scores, highlighting calcium's critical role in musculoskeletal and neuromuscular functions. Adequate calcium levels contribute to better muscle function and reduced risks of muscular weakness, as reflected in improved physical function scores. However, serum calcium's association with ISI scores was minimal, suggesting that calcium levels do not significantly impact sleep-related outcomes. This underscores vitamin D's unique role in sleep health, distinguishing it from calcium's primary influence on physical well-being.

Significant correlations between SF-36 physical health parameters—specifically physical functioning and general health—and ISI scores were observed, with lower physical functioning scores associated with higher ISI scores, indicating more severe insomnia in individuals experiencing greater physical limitations. This relationship underscores the interdependent nature of physical impairments and sleep disturbances, as physical limitations may exacerbate the severity of insomnia. Additionally, the significant association between general health perceptions and ISI scores suggests that individuals who perceive poorer overall health also report more severe insomnia, emphasizing how physical and mental health collectively impact sleep quality.

The correlations observed between vitamin D levels, HRQoL, and ISI scores may be partially explained by vitamin D's involvement in hormonal and neurological regulation. Vitamin D interacts with vitamin D receptors (VDRs), influencing gene expression related to essential hormones and neurotransmitters that maintain physical and mental health. Specifically, VDR activation promotes serotonin and melatonin synthesis, both of which play critical roles in mood regulation and sleep maintenance. Low vitamin D levels can interfere with this regulatory process, potentially disrupting circadian rhythms and aggravating insomnia. Additionally, vitamin D influences melatonin production by binding to receptors in the hypothalamus, which governs circadian alignment; insufficient vitamin D can lead to circadian misalignment and exacerbate sleep disorders, including insomnia and obstructive sleep apnea syndrome (OSAS).

Vitamin D deficiency may also intensify immune and inflammatory responses by elevating pro-inflammatory cytokines, such as IL-6 and TNF-α, which are associated with disrupted sleep patterns

TABLE 5 Correlation of vitamin D and serum calcium levels with SF-36 (Physical, mental health) and ISI score.

Variables	SF-36										ISI
	Physical Health					Mental Health					
	Variables	Physical Function	Role Physical	Body Pain	General Health	PCS	Vitality	Social Functioning	Role Emotional	Mental Health	MCS	
Vitamin D	Pearson correlation coefficient	0.642	0.054	0.011	0.560	0.441	0.018	0.096	0.000	−0.01	0.012	−0.112
	*p value**	**0.000**	0.235	0.806	**0.000**	**0.000**	0.690	**0.035**	0.995	0.822	0.800	**0.014**
Serum calcium	Pearson correlation coefficient	0.008	0.050	0.040	0.185	0.018	0.007	0.006	−0.051	−0.045	0.054	0.031
	*p value**	**0.000**	0.276	0.377	**0.021**	**0.047**	0.876	0.894	0.267	0.324	0.235	0.495

*The correlation is significant at p value < 0.05.

TABLE 6 Correlation between SF-36 (Physical, mental health) and ISI score.

ISI	SF-36									
	Physical health					Mental health				
	Variables	Physical function	Role physical	Body pain	General health	PCS	Vitality	Social functioning	Role emotional	Mental health	MCS
	Pearson Correlation Coefficient	−0.193	−0.015	−0.026	−0.107	−0.096	0.020	−0.189	0.075	0.034	0.045
	*p value**	**0.000**	0.745	0.526	**0.018**	**0.035**	0.665	**0.000**	0.100	0.460	0.326

*The correlation is significant at p value < 0.05.

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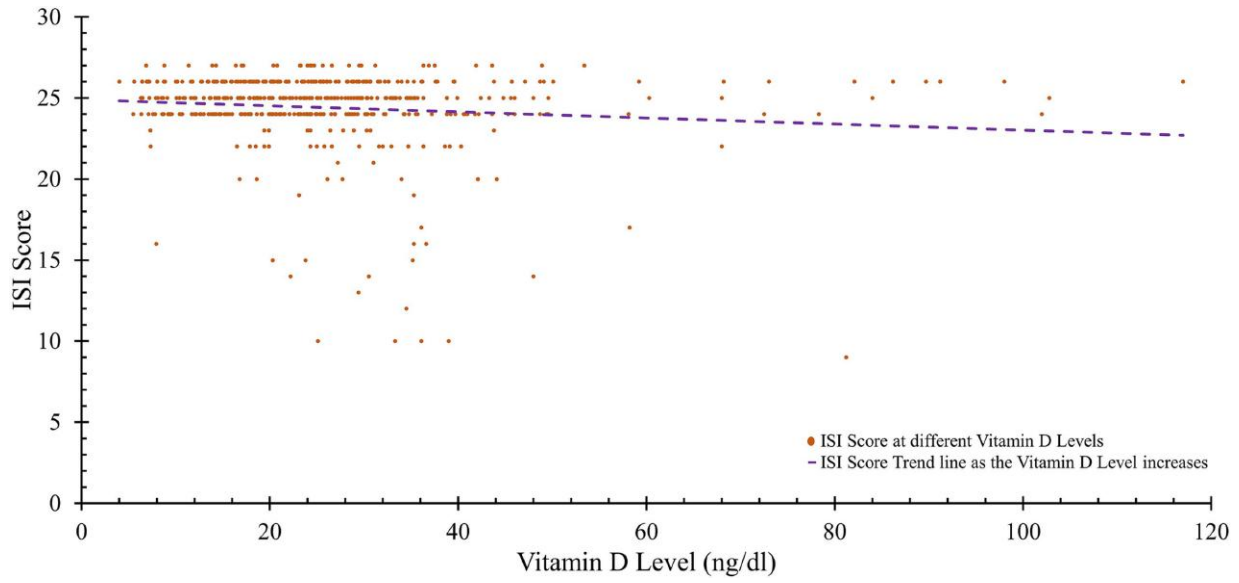

FIGURE 2
Insomnia severity index of subjects with varying vitamin D levels.

and poor sleep quality. This inflammatory response may lead to fragmented sleep and worsen airway obstruction, as seen in OSAS (48). Furthermore, vitamin D modulates the body's hypoxic response via its influence on HIF-1α, potentially mitigating sleep disturbances related to hypoxic episodes. These physiological pathways suggest a complex and multifactorial role of vitamin D in maintaining sleep quality, providing further insights into the observed associations between vitamin D, HRQoL, ISI scores, and overall health (49, 50).

These findings underscore the importance of vitamin D assessments in public health and clinical settings to improve both physical and mental health outcomes. Positive correlations between vitamin D, HRQoL, and insomnia severity suggest that vitamin D status is a modifiable factor with potential to enhance overall quality of life, particularly for populations with high vitamin D deficiency rates. Integrating vitamin D screening and supplementation into healthcare practices may help reduce the burden of sleep disorders and physical impairments, contributing to improved HRQoL and well-being across affected populations. Furthermore, this study contributes to the existing literature by examining vitamin D's broader impact, not only on physical health but also on sleep quality, mental well-being, and social functioning. This study provides novel insights into vitamin D's relationship with insomnia severity and social engagement, offering a more comprehensive understanding of vitamin D's influence on overall HRQoL.

Additionally, the focus on an Indian population with distinct cultural and dietary practices adds valuable perspectives to the global research on vitamin D, highlighting region-specific health interventions and preventive care strategies.

4.1 Limitations of the study

The study's 6-month duration may have limited its ability to fully capture the long-term effects of vitamin D on physical health, mental well-being, and sleep quality. Recruitment challenges due to specific selection criteria and the single-center design further restrict the generalizability of the findings to broader populations or different settings. Additionally, direct measures of sunlight exposure—a key factor in vitamin D synthesis—were not included, potentially affecting the study's accuracy regarding vitamin D levels and related health outcomes. Future studies would benefit from incorporating direct sunlight measurements to provide a clearer understanding of its impact. Notably, the correlation coefficients for physical and mental health and insomnia were found to be close to or slightly higher than those for insomnia and vitamin D levels, suggesting that the observed effects on sleep quality may arise not only from vitamin D's direct influence on sleep biochemistry, such as melatonin synthesis, but also through secondary effects on physical and mental health. Expanding future research to longitudinal, multi-site studies with diverse populations could further clarify these relationships, enhancing the generalizability and causal understanding of vitamin D's role in HRQoL, insomnia severity, and overall health metrics.

5 Conclusion

The findings of this study underscore a meaningful connection between higher levels of vitamin D and improved physical and mental well-being, as indicated by better performance on the SF-36 mental health scale and the Physical Component Summary (PCS). Notably, we also observed a significant negative relationship between vitamin D levels and insomnia severity, suggesting that adequate vitamin D may play a vital role in enhancing overall health and well-being. Given these results, there is a pressing need for initiatives aimed at reducing vitamin D deficiency. Additionally, further research is essential to deepen our understanding of the specific mechanisms that link vitamin D to a diverse range of health outcomes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Institutional Ethics Committee (IEC) at NIMS University Rajasthan. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AS: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. SK: Data curation, Formal analysis, Investigation, Writing – original draft. SM: Formal analysis, Methodology, Visualization, Writing – original draft. SR: Formal analysis, Investigation, Visualization, Writing – original draft. SD: Formal analysis, Methodology, Writing – original draft. PR: Conceptualization, Supervision, Validation, Writing – review & editing. HB: Resources, Supervision, Validation, Writing – review & editing. MS: Conceptualization, Supervision, Validation, Writing – review & editing. DN: Project administration, Resources, Software, Supervision, Writing – review & editing. BT: Resources, Software, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Vitamin D supplementation may be beneficial in improving the prognosis of patients with sepsis-associated acute kidney injury in the intensive care unit: a retrospective study

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Background: Vitamin D, an essential fat-soluble micronutrient, exerts diverse physiological effects including the regulation of calcium ion homeostasis, modulation of immune response, and enhancement of resistance against infectious pathogens. Empirical investigations have elucidated an association between inadequate levels of vitamin D and adverse clinical outcomes in critically ill cohorts, with a noteworthy prevalence of vitamin D deficiency observed among patients afflicted with acute kidney injury (AKI). In the context of this retrospective inquiry, our aim was to assess the potential correlation between vitamin D supplementation administered during admission to the intensive care unit (ICU) and the improvement of outcomes specifically in cases of severe AKI.

Methods: This study utilized data from the Medical Information Mart for Intensive Care IV (MIMIC-IV), a repository of ICU patient records from Beth Israel Deaconess Medical Center (BIDMC) in the United States. We focused on patients diagnosed with epsis-associated acute kidney injury (SA-AKI), dividing them into those who received vitamin D supplementation during their ICU admission and those who did not. Our primary analysis evaluated in-hospital mortality using various statistical methods, such as Kaplan–Meier survival curves, Cox proportional hazards regression models, and subgroup analyses. To enhance the robustness of our findings, we used propensity score matching (PSM) to reduce potential biases. Secondary outcomes included 28-day, 90-day mortality rates and norepinephrine-free days at 28 days.

Results: In this investigation, a cohort of 11,896 individuals diagnosed with SA-AKI was studied. Among them, 2,724 patients received vitamin D supplementation (the vitamin D group) while 9,172 did not (the no-vitamin D group). Kaplan–Meier survival analysis indicated a significant difference in survival probabilities between the two cohorts. Upon adjusting for potential confounders using Cox regression modeling, a notably decreased risk of hospitalization and ICU mortality was observed in the vitamin D group compared to the no-vitamin D group, with an adjusted risk ratio for in-hospital mortality of 0.56 (95% CI: 0.5–0.63). These findings were consistent following PSM and subsequent adjustments for propensity score, pairwise algorithm (PA), and overlapping weights (OW) analyses, yielding hazard ratios ranging from 0.53 to 0.59, all with

p-values <0.001. Notably, E-value analyses underscored the robustness of these results against potential unmeasured confounders.

Conclusion: This study suggests that vitamin D supplementation may be associated with a reduced in-hospital mortality rate among SA-AKI patients in the ICU. Furthermore, the 28-day, 90-day mortality rates and norepinephrine days were significantly reduced in the group receiving vitamin D supplementation.

KEYWORDS

vitamin D supplementation, mortality, SA-AKI, MIMIC-IV, intensive care unit

1 Introduction

Sepsis is defined as a state of organ dysfunction resulting from the dysregulation of the host's immune response to infection, thus posing a considerable risk of morbidity and mortality in critically ill patients (1). Notably, the kidney assumes a primary and early role in sepsis, with acute kidney injury (AKI) being a comprehensive clinical syndrome characterized by a sudden decline in renal function, encompassing various manifestations beyond the scope of acute renal failure alone (2). Importantly, the occurrence of sepsis-associated acute kidney injury (SA-AKI) significantly escalates the likelihood of in-hospital mortality, the development of chronic kidney disease, and the need for renal replacement therapy (3, 4).

Studies have elucidated the pleiotropic effects of vitamin D on immune function, endothelial and mucosal integrity, and glucose metabolism, in addition to its established role in calcium homeostasis regulation (5). Furthermore, numerous investigations have underscored the associations between vitamin D deficiency and heightened mortality and morbidity rates across diverse chronic conditions, encompassing coronary artery disease, tuberculosis, malignant neoplasms, and chronic kidney disease (6).

In the context of critically ill patients, vitamin D insufficiency has been correlated with a significantly increased incidence of sepsis and organ dysfunction, both of which are implicated in elevated mortality rates (7, 8). However, despite an extensive corpus of literature interrogating the nexus between vitamin D and sepsis in critically ill cohorts, scant attention has been devoted to investigating SA-AKI. Remarkably, the biologically active form of vitamin D is synthesized within the renal proximal tubule mitochondria, where 1α-hydroxylase catalyzes the conversion of 25-OH vitamin D to its biologically active metabolite, 1,25-dihydroxy vitamin D (9). The principal mechanism underlying SA-AKI involves renal ischemia–reperfusion injury. Interestingly, evidence suggests that the severity of this injury correlates with deficiencies in vitamin D receptor expression and the downregulation of P21 (10). The primary aim of this study was to assess the potential efficacy of vitamin D supplementation in improving clinical outcomes among intensive care unit (ICU) SA-AKI patients.

2 Methods

2.1 Data sources and setting

A population-based cohort study utilized the Critical Care Database from the Medical Information Mart for Intensive Care (MIMIC-IV, version 2.2), an extension of MIMIC-III. This database encompassed 76,540 ICU admissions spanning 2008 to 2019. Approval to access the database (certification number 54835759) was obtained by Jie Sun. Data were de-identified prior to use, and both the institutional review boards of Massachusetts Institute of Technology (No. 0403000206) and Beth Israel Deaconess Medical Center (2001-P-001699/14) approved its utilization for research. Strict adherence to ethical regulations governing research data use was maintained throughout the study.

2.2 Study population

A total of 50,920 patients who were admitted to the ICU for the first time were identified from the MIMIC database. The flow chart and number of patients in this study are shown in Figure 1. Only patients diagnosed with SA-AKI were included. The study excluded individuals with a hospitalization duration of less than 48 h and those under 18 years of age. The study subjects conformed to the sepsis-3 criteria from the Third International Consensus for diagnosing sepsis and septic shock (11). AKI identification and classification followed the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, AKI was defined as a serum creatinine (sCr) increase of ≥0.3 mg/dL (26.5 μmol/L) within 48 h, sCr elevation to ≥1.5 times baseline in the past 7 days, or urine output <0.5 mL/kg/h over a 6-h period (12). If the baseline sCr level was not documented before ICU admission, the first recorded sCr level post-admission was used as the baseline reference.

2.3 Main exposure

The primary independent variable under consideration was the administration of vitamin D supplementation (via both intravenous and oral routes) after admission to the ICU, which led to categorizing patients into a vitamin D cohort and a non-vitamin D cohort.

2.4 Covariates

In this study, we measured patient characteristics that have previously been demonstrated to influence changes in mortality rates of SA-AKI. The variables examined in this study included demographic factors such as age, gender, and race, alongside vital signs and a comprehensive range of laboratory tests. Vital signs were recorded as average values within the first 24 h post-ICU admission, while laboratory indicators were derived from the worst values observed during the same period. The laboratory tests encompassed

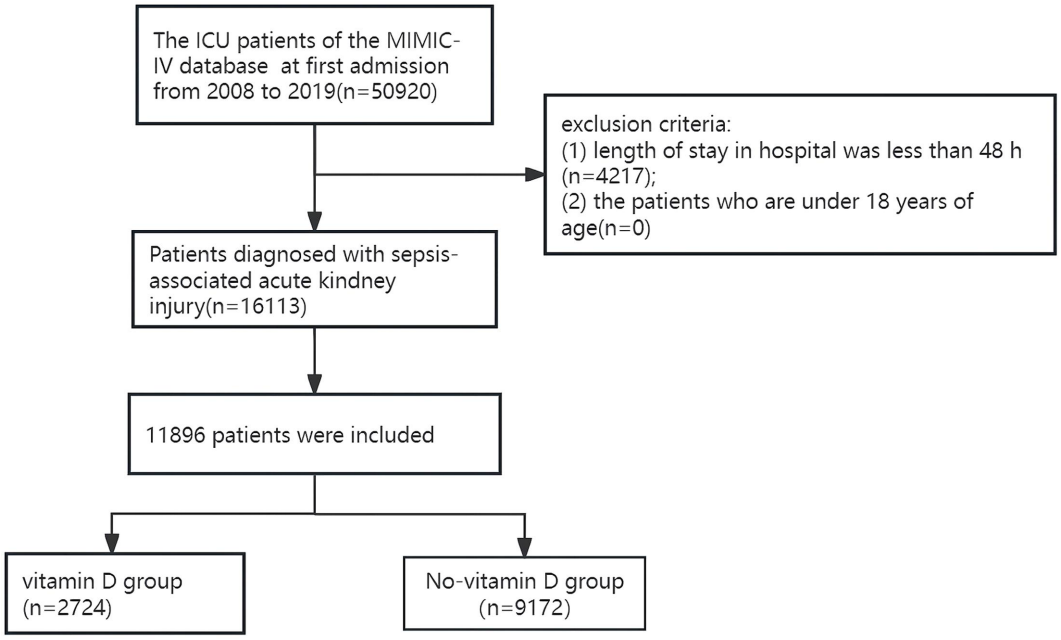

FIGURE 1
Flowchart of participant selection.

parameters including hemoglobin, white blood cell (WBC) count, platelet count, bicarbonate, creatinine, sodium, potassium, calcium, chloride, blood urea nitrogen (BUN), prothrombin time (PT), activated partial thromboplastin time (APTT), serum lactate, and glucose. Additionally, comorbidities including myocardial infarction, congestive heart failure, rheumatic disease, renal disease, chronic pulmonary disease, peptic ulcer disease, mild liver disease, severe liver disease, malignant cancer, hypertension, and diabetes mellitus were recorded, alongside the charlson comorbidity index. AKI staging was determined in accordance with the KDIGO criteria. Throughout the treatment process, the use of invasive mechanical ventilation, vasoactive drugs, renal replacement therapy (RRT) and disease severity scores, such as the Sequential Organ Failure Assessment (SOFA) Score and the Simplified Acute Physiology Score (SAPS) II, were documented, we used the SOFA score and SAPS II score from the first day after admission to the ICU as assessment indicators.

2.5 Primary outcome and secondary outcomes

The primary outcome of the study was in-hospital mortality, Secondary outcomes include 28-day mortality, 90-day mortality and norepinephrine-free days within 28 days after ICU admission.

2.6 Statistical analysis

Descriptive analyses were performed for all participants. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as means with standard deviations (SD) for normally distributed data, or medians with interquartile ranges (IQR) for skewed data. Statistical tests included the chi-square test for categorical variables, the *t*-test for normally distributed continuous variables, and the Kruskal-Wallis test for non-normally distributed continuous variables. Kaplan–Meier estimates and log-rank tests were employed to analyze survival curves.

To minimize potential bias resulting from confounding factors among various groups, we calculated propensity scores using logistic regression and applied a 1:1 nearest neighbor matching algorithm with a caliper width of 0.01. The variables selected for this analysis included age, sex, race, vital signs, laboratory test results, comorbidities, and levels of disease verification, as informed by the existing literature. The standardized mean difference (SMD) was utilized to assess the effectiveness of the propensity score matching, with a threshold of less than 0.1 considered acceptable.

In the propensity score matched (PSM) cohort, we conducted a two-sided *t*-test to evaluate differences in the secondary outcome between the two groups. To investigate the relationship between vitamin D supplementation and in-hospital mortality, we performed both univariate and multivariate Cox regression analyses, with the multivariate analysis incorporating all variables used in generating the propensity scores. The estimated propensity scores were utilized as weights to adjust for intergroup differences. A weighted cohort was created using the pairwise algorithm (PA) (13) and overlapping weights (OW) (14) models.

All analyses were carried out using the statistical software package R version 3.3.2[1] and the free statistical software version 1.4 of R (15). Two-tailed tests were performed on www.R-project.org, with *p*-values less than 0.05 regarded as statistically significant.

1 http://www.example.com, R Foundation.

2.7 Sensitivity analysis and subgroup analysis

We performed a sensitivity analysis on patients who developed AKI within the 48-h period preceding ICU admission. Furthermore, we conducted subgroup analyses categorized by age, sex, SOFA score, SAPS II score, use of vasoactive medications, presence of invasive ventilation, as well as comorbid conditions including hypertension, diabetes mellitus, renal disease, chronic pulmonary disease, and congestive heart failure. The possibility of unmeasured confounders between the vitamin D supplementation group and in-hospital mortality was assessed by calculating E-values (16).

3 Results

3.1 Participants

A total of 50,920 patients who were admitted to the ICU for the first time were identified from the MIMIC database. The flow chart and number of patients in this study are shown in Figure 1. Only patients diagnosed with SA-AKI were included. Excluded from the study were patients who had a hospital stay in the ICU for less than 48 h, and patients who were under 18 years old. A total of 11,896 patients with SA-AKI were included in the study, among which 2,724 patients received vitamin D supplementation, and 9,172 patients did not receive vitamin D supplementation.

3.2 Baseline characteristics

The baseline characteristics of all subjects are presented in Table 1. The mean age of the participants was 67.1 ± 16.0 years, with 57.7% (6,862 individuals) being male. BUN levels were elevated in the vitamin D supplementation group. Additionally, there was a higher prevalence and increased Charlson comorbidity index for conditions such as congestive heart failure, chronic pulmonary disease, rheumatic disease, severe liver disease, diabetes mellitus, hypertension, renal disease comorbidities, and malignant cancer in this group. Furthermore, the odds of requiring RRT during admission were greater in the vitamin D supplementation group, whereas the need for mechanical ventilation during admission was more prevalent in the group that did not receive vitamin D supplementation. The baseline characteristics of the two groups after PSM are balanced in Table 1.

3.3 Primary outcome

The overall in-hospital mortality rate was 19.2%, and the in-hospital mortality rates for the vitamin D-supplemented group and the non-vitamin D-supplemented group were 14% (381/2724) and 20.7% (1899/9172), respectively (Figure 2) and the Kaplan–Meier curves showed that the vitamin D-supplemented group had a lower rate of in-hospital mortality (Figure 3).

In this study, univariate Cox regression analysis demonstrated that vitamin D supplementation significantly reduces the mortality risk in patients with SA-AKI, yielding a hazard ratio (HR) of 0.59 (95% CI: 0.52–0.65). This finding indicates that patients receiving vitamin D have an approximately 41% lower risk of death compared to those who do not receive supplementation. To account for potential confounding factors, we adjusted for all covariates listed in Table 1 during the multivariable Cox regression analysis, which resulted in a hazard ratio of 0.56 (95% CI: 0.50–0.63), suggesting the robustness of the results. Furthermore, we utilized PSM and adjusted for the propensity score, to mitigate the influence of confounders. The results revealed consistent hazard ratios of 0.56 (95% CI: 0.50–0.63) and 0.53 (95% CI: 0.47–0.61), reinforcing the assertion that vitamin D supplementation has a stable effect on reducing mortality risk. Lastly, the incorporation of the PA and OW methods further bolstered the reliability of our findings (see Figure 2). Notably, the E-value for this cohort ranged from 2.78 to 3.18, indicating a robust association between vitamin D supplementation and improved patient outcomes, thereby suggesting that this association remains significant even in the presence of potential unmeasured confounders.

3.4 Secondary outcome analysis with PSM cohorts

After adjusting for confounders using PSM and comparing the 28-day and 90-day mortality rates between the two groups, a statistically significant reduction in mortality was observed in patients who received vitamin D supplementation (16.5% vs. 26.3%, $p < 0.001$; 23.3% vs. 34.7%, $p < 0.001$). Furthermore, the vitamin D supplementation group exhibited a higher number of vasopressor-free days (21.7 ± 10.5 vs. 19.6 ± 11.9, $p < 0.001$) (Table 2).

3.5 Sensitivity analysis and subgroup analysis

We performed a sensitivity analysis on patients who developed AKI within 48 h of ICU admission. Utilizing univariate and multivariable Cox regression analyses, PSM, adjusted for propensity score, PA, and OW methods, we found that vitamin D supplementation significantly reduces mortality rates among these patients (Table 3).

After adjusting for all covariates in Table 1, we conducted a subgroup analysis based on age, gender, SOFA score, SAPS II score, the use of vasopressor agents, the presence of invasive mechanical ventilation, and the existence of hypertension, diabetes, renal disease, chronic pulmonary disease, and congestive heart failure. The results remained stable across these subgroups. However, some interactions were observed concerning age, SOFA scores, and mechanical ventilation (Figure 4).

4 Discussion

Our study suggests that vitamin D supplementation during hospital admission is associated with a lower risk of in-hospital mortality in patients with SA-AKI. This association was further validated by PSM, PA, OW, sensitivity analysis and subgroup analyses. E-value analyses ranging from 2.78 to 3.18 indicate that unmeasured confounders are unlikely to negate the observed effects. The results confirm that vitamin D, as an inexpensive, readily available and relatively safe intervention, is associated with improved prognosis in SA-AKI, demonstrating the robustness and reliability of the findings. In addition, our findings suggest that vitamin D supplementation during hospitalization is not

TABLE 1 Baseline characteristics of participants.

Covariate	Unmatched patients			SMD	*p* value	Propensity-score–matched patients			SMD	*p* value
	Total	No vitamin D	Vitamin D			Total	No vitamin D	Vitamin D		
n	11,896	9,172	2,724			5,392	2,696	2,696		
Age (years)	67.1 ± 16.0	66.3 ± 16.2	69.9 ± 14.7	0.234	< 0.001	69.9 ± 14.7	69.9 ± 14.8	70.0 ± 14.7	0.003	0.901
Male, sex, *n* (%)	6,862 (57.7)	5,523 (60.2)	1,339 (49.2)	0.224	< 0.001	2,654 (49.2)	1,319 (48.9)	1,335 (49.5)	0.012	0.663
Race, *n* (%)				0.361	< 0.001				0.026	0.921
White	7,851 (66.0)	5,890 (64.2)	1961 (72)			3,916 (72.6)	1972 (73.1)	1944 (72.1)		
Black	955 (8.0)	636 (6.9)	319 (11.7)			606 (11.2)	298 (11.1)	308 (11.4)		
Asia	284 (2.4)	210 (2.3)	74 (2.7)			142 (2.6)	68 (2.5)	74 (2.7)		
Hispanic	261 (2.2)	204 (2.2)	57 (2.1)			109 (2.0)	52 (1.9)	57 (2.1)		
Other	2,545 (21.4)	2,232 (24.3)	313 (11.5)			619 (11.5)	306 (11.4)	313 (11.6)		
Vital Signs, mean (SD)										
Heart rate (bpm)	87.5 ± 16.6	87.7 ± 16.6	86.9 ± 16.8	0.051	0.019	87.0 ± 16.5	87.2 ± 16.1	86.9 ± 16.8	0.019	0.496
MAP (mmHg)	76.6 ± 10.2	77.0 ± 10.3	75.5 ± 9.9	0.141	< 0.001	75.4 ± 9.9	75.2 ± 10.0	75.5 ± 9.8	0.034	0.218
Respiratory rate (bpm)	19.9 ± 4.1	19.9 ± 4.1	19.9 ± 4.1	0.02	0.35	19.9 ± 4.1	19.9 ± 4.1	19.9 ± 4.1	0.003	0.908
Temperature (°C)	36.9 ± 0.7	36.9 ± 0.7	36.8 ± 0.6	0.085	< 0.001	36.8 ± 0.6	36.8 ± 0.6	36.9 ± 0.6	0.01	0.709
SPO2 (%)	97.1 ± 2.2	97.1 ± 2.2	96.9 ± 2.2	0.081	< 0.001	96.9 ± 2.2	96.9 ± 2.2	96.9 ± 2.2	0.004	0.878
Laboratory tests										
Hemoglobin, g/dL	9.9 ± 2.2	10.0 ± 2.2	9.4 ± 2.1	0.264	< 0.001	9.4 ± 2.1	9.4 ± 2.1	9.4 ± 2.1	0.006	0.828
Platelets, 10^9/L	160.0 (109.0, 225.0)	161.0 (111.0, 224.0)	158.5 (103.0, 228.0)	0.015	0.094	158.0 (104.0, 227.0)	156.0 (105.0, 226.0)	159.0 (103.0, 228.0)	0.006	0.834
White Blood Cell,10^9/L	14.4 (10.4, 19.4)	14.6 (10.6, 19.6)	13.8 (9.7, 18.9)	0.03	< 0.001	13.8 (9.9, 19.0)	13.9 (10.2, 19.0)	13.8 (9.7, 18.9)	0.005	0.225
Bicarbonate, mmol/L	24.1 ± 4.5	24.1 ± 4.4	24.2 ± 4.8	0.005	0.8	24.2 ± 4.7	24.2 ± 4.6	24.2 ± 4.8	0.002	0.929
BUN, mg/dL	24.0 (17.0, 40.0)	23.0 (16.0, 38.0)	29.0 (19.0, 47.0)	0.239	< 0.001	28.0 (18.0, 47.0)	28.0 (18.0, 48.0)	29.0 (18.0, 47.0)	0.01	0.723
Calcium, mmol/L	8.5 ± 0.9	8.5 ± 0.9	8.6 ± 1.0	0.113	< 0.001	8.6 ± 1.0	8.6 ± 0.9	8.6 ± 1.0	0.007	0.79
Chloride, mmol/L	106.4 ± 6.8	106.7 ± 6.7	105.6 ± 7.0	0.164	< 0.001	105.6 ± 7.0	105.6 ± 7.0	105.6 ± 7.0	0.007	0.795
Sodium, mmol/L	140.2 ± 5.4	140.3 ± 5.4	139.7 ± 5.5	0.101	< 0.001	139.7 ± 5.5	139.7 ± 5.5	139.8 ± 5.5	0.01	0.718
Potassium, mmol/L	4.7 ± 0.9	4.7 ± 0.9	4.7 ± 0.9	0.056	0.009	4.7 ± 0.9	4.8 ± 0.9	4.7 ± 0.9	0.018	0.504
Serum creatinine, mg/dL	1.2 (0.9, 2.0)	1.2 (0.9, 1.9)	1.3 (0.9, 2.3)	0.175	< 0.001	1.3 (0.9, 2.3)	1.3 (0.9, 2.4)	1.3 (0.9, 2.3)	0.02	0.195
Glucose, mmol/L	152.0 (122.0, 203.0)	151.0 (122.0, 201.0)	154.0 (122.0, 211.0)	0.057	0.008	154.0 (122.0, 211.0)	154.0 (122.0, 211.0)	154.0 (123.0, 211.0)	0.006	0.848
PT, s	15.1 ± 6.1	14.9 ± 5.7	15.9 ± 7.3	0.156	< 0.001	15.9 ± 7.0	15.9 ± 7.1	15.8 ± 6.9	0.013	0.763
APTT, s	31.8 ± 11.1	31.5 ± 10.7	33.0 ± 12.4	0.13	< 0.001	33.0 ± 12.7	33.1 ± 13.1	32.8 ± 12.2	0.02	0.764

(Continued)

TABLE 1 (Continued)

Covariate	Unmatched patients			SMD	*p* value	Propensity-score–matched patients			SMD	*p* value
	Total	No vitamin D	Vitamin D			Total	No vitamin D	Vitamin D		
Serum lactate, mmol/L	2.3 (1.5, 3.6)	2.3 (1.5, 3.6)	2.2 (1.4, 3.7)	0.027	0.027	2.2 (1.4, 3.6)	2.2 (1.5, 3.5)	2.2 (1.4, 3.6)	<0.001	0.559
Comorbidities, *n* (%)										
Myocardial infarction, *n* (%)	2,299 (19.3)	1775 (19.4)	524 (19.2)	0.003	0.893	1,060 (19.7)	537 (19.9)	523 (19.4)	0.013	0.631
Congestive heart failure, *n* (%)	4,019 (33.8)	2,929 (31.9)	1,090 (40)	0.169	< 0.001	2,141 (39.7)	1,064 (39.5)	1,077 (39.9)	0.01	0.717
Chronic pulmonary disease, *n* (%)	3,326 (28.0)	2,492 (27.2)	834 (30.6)	0.076	< 0.001	1,652 (30.6)	830 (30.8)	822 (30.5)	0.006	0.813
Rheumatic disease, *n* (%)	432 (3.6)	255 (2.8)	177 (6.5)	0.177	< 0.001	325 (6.0)	162 (6)	163 (6)	0.002	0.954
Peptic ulcer disease, *n* (%)	366 (3.1)	282 (3.1)	84 (3.1)	0.001	0.981	173 (3.2)	90 (3.3)	83 (3.1)	0.015	0.589
Mild liver disease, *n* (%)	1885 (15.8)	1,372 (15)	513 (18.8)	0.104	< 0.001	995 (18.5)	495 (18.4)	500 (18.5)	0.005	0.861
Severe liver disease, *n* (%)	940 (7.9)	630 (6.9)	310 (11.4)	0.157	< 0.001	597 (11.1)	300 (11.1)	297 (11)	0.004	0.896
Diabetes, *n* (%)	3,753 (31.5)	2,717 (29.6)	1,036 (38)	0.178	< 0.001	2070 (38.4)	1,037 (38.5)	1,033 (38.3)	0.003	0.911
Hypertension, *n* (%)	6,489 (54.5)	4,842 (52.8)	1,647 (60.5)	0.155	< 0.001	3,233 (60.0)	1,607 (59.6)	1,626 (60.3)	0.014	0.597
Renal disease, *n* (%)	2,792 (23.5)	1904 (20.8)	888 (32.6)	0.27	< 0.001	1739 (32.3)	871 (32.3)	868 (32.2)	0.002	0.93
Malignant cancer, *n* (%)	1,552 (13.0)	1,168 (12.7)	384 (14.1)	0.04	0.064	751 (13.9)	369 (13.7)	382 (14.2)	0.014	0.609
Charlson comorbidity index	6.1 ± 2.9	5.9 ± 2.9	6.7 ± 2.7	0.304	< 0.001	6.7 ± 2.7	6.7 ± 2.7	6.7 ± 2.8	0.02	0.537
Scoring system										
SAPS II	42.5 ± 14.2	42.2 ± 14.3	43.7 ± 13.7	0.108	< 0.001	43.6 ± 13.7	43.6 ± 13.8	43.6 ± 13.7	0.002	0.941
SOFA	3.0 (2.0, 5.0)	3.0 (2.0, 5.0)	3.0 (2.0, 5.0)	0.071	< 0.001	3.0 (2.0, 5.0)	3.0 (2.0, 5.0)	3.0 (2.0, 5.0)	0.005	0.857
AKI stage, *n* (%)				0.115	< 0.001				0.025	0.862
1	2,269 (19.1)	1811 (19.7)	458 (16.8)			904 (16.8)	447 (16.6)	457 (17)		
2	5,658 (47.6)	4,408 (48.1)	1,250 (45.9)			2,459 (45.6)	1,218 (45.2)	1,241 (46)		
3	3,969 (33.4)	2,953 (32.2)	1,016 (37.3)			2029 (37.6)	1,031 (38.2)	998 (37)		
GCS	10.7 ± 4.2	10.5 ± 4.2	11.2 ± 3.9	0.155	0.001	11.2 ± 3.9	11.1 ± 4.0	11.2 ± 3.9	0.007	0.798
RRT	1,550 (13.0)	1,105 (12)	445 (16.3)	0.123	0.001	873 (16.2)	446 (16.5)	427 (15.8)	0.019	0.482
Vasopressors use, *n* (%)	1,400 (11.8)	1,052 (11.5)	348 (12.8)	0.04	0.063	694 (12.9)	353 (13.1)	341 (12.6)	0.013	0.626
Invasive ventilation, *n* (%)	8,216 (69.1)	6,588 (71.8)	1,628 (59.8)	0.256	0.001	3,239 (60.1)	1,628 (60.4)	1,611 (59.8)	0.013	0.636

MAP, Mean Arterial Pressure; SPO2, pulse oxygen saturation; AKI, acute kidney injury; BUN, blood urea nitrogen; SOFA, sequential organ failure assessment; SAPS II, simplified acute physiology score II; RRT, Renal Replacement Therapy; GCS, Glasgow Coma Scale; PT, Prothrombin Time; APTT, Activated Partial Thromboplastin Time.

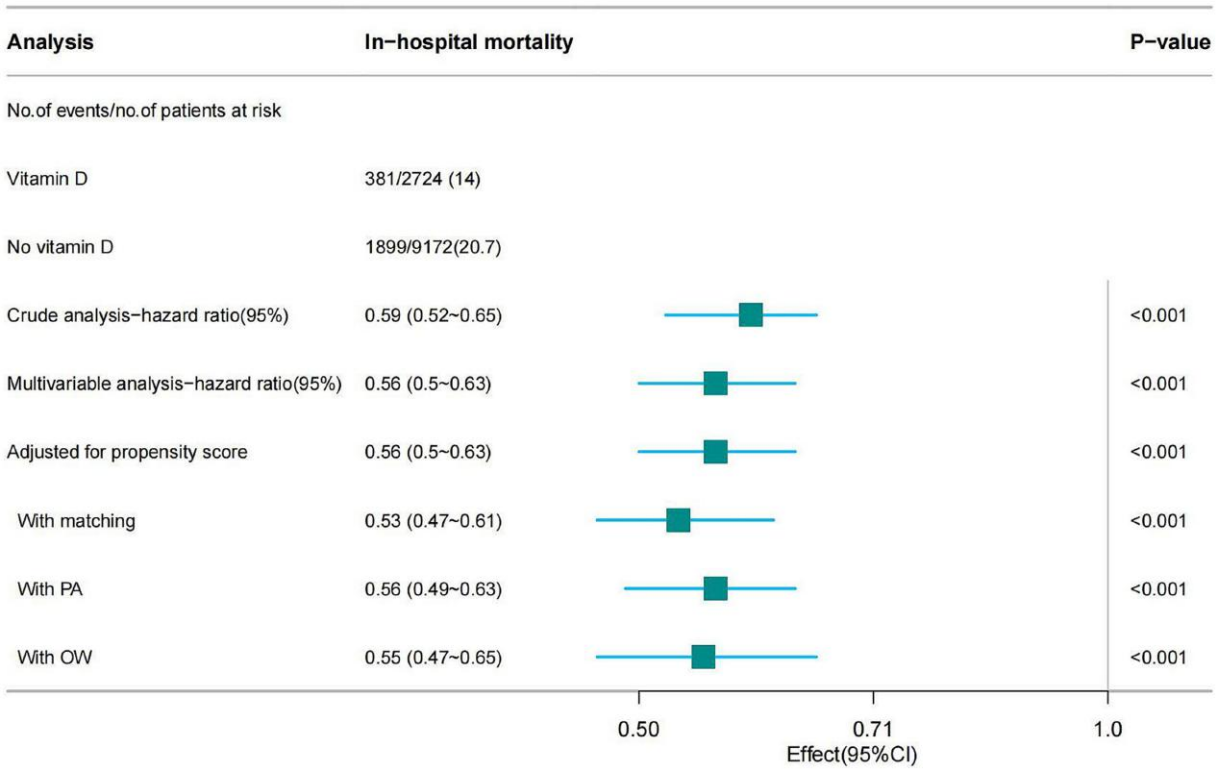

FIGURE 2
Forest plot shows HRs of in-hospital mortality in vitamin D group using a variety of models.

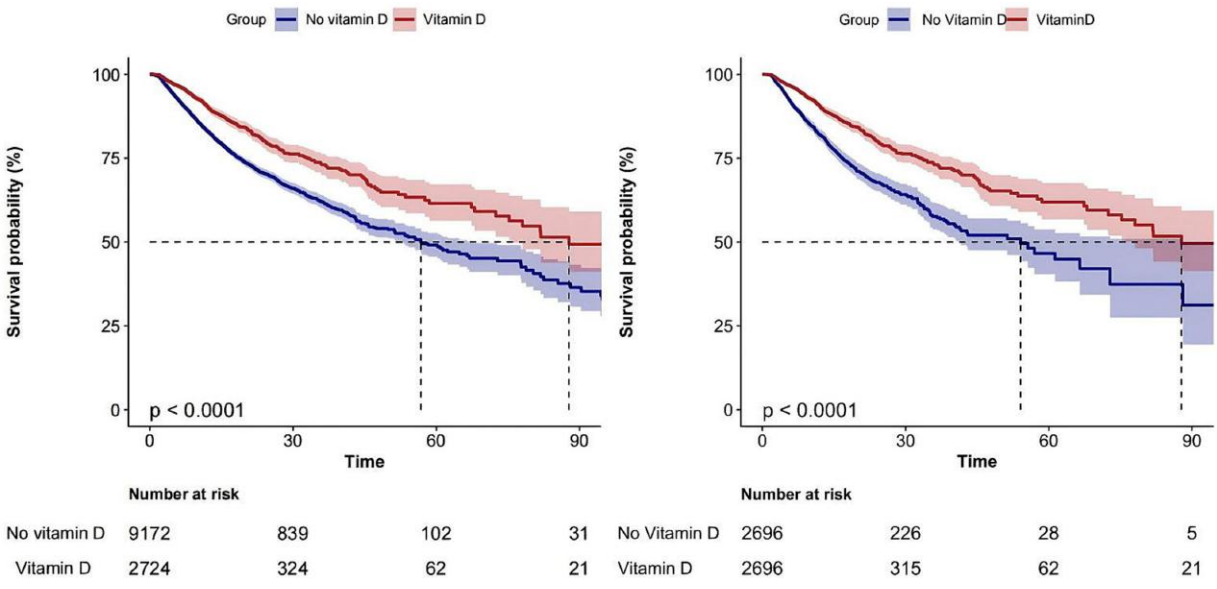

FIGURE 3
Kaplan–Meier survival curve for in-hospital mortality according to different groups.

only associated with reduced in-hospital mortality, but also with reduced 28-day and 90-day mortality. At the same time, patients receiving vitamin D supplementation had a higher number of days without norepinephrine. These results provide further evidence to support the beneficial role of vitamin D in the treatment of SA-AKI.

The body uses two main forms of vitamin D: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). These forms are obtained from the diet and synthesized in the skin. They are metabolized in the liver to form 25-hydroxyvitamin D. Subsequently, the enzyme 25-hydroxyvitamin D-1alpha-hydroxylase catalyzes its

TABLE 2 Secondary outcome analysis after matching.

Secondary outcomes	Total	Propensity-score–matched patients		*p*-value
		No vitamin D	Vitamin D	
28-day mortality, *n* (%)	1155/5390 (21.4)	710/2695 (26.3)	445/2695 (16.5)	< 0.001
90-day mortality, *n* (%)	1564/5394 (29.0)	936/2697 (34.7)	628/2697 (23.3)	< 0.001
Norepinephrine free day, Mean (SD)	5,394	19.6 ± 11.9	21.7 ± 10.5	< 0.001

TABLE 3 HRs of in-hospital mortality in vitamin D group using a variety of models.

Analysis	In-hospital mortality	*p*-value
No. of events/no. of patients at risk		
Vitamin D	350/2140 (14.1)	
No vitamin D	1728/8178 (21.1)	
Crude analysis-hazard ratio (95%)	0.57 (0.51 ~ 0.64)	<0.001
Multivariable analysis-hazard ratio (95%)	0.54 (0.47 ~ 0.6)	<0.001
Adjusted for propensity score	0.54 (0.48 ~ 0.61)	<0.001
With matching	0.54 (0.47 ~ 0.62)	<0.001
With PA	0.54 (0.47 ~ 0.62)	<0.001
With OW	0.53 (0.45 ~ 0.63)	<0.001

conversion in the kidneys to the active form, 1,25-dihydroxyvitamin D (6, 17). Although vitamin D has historically been associated primarily with skeletal metabolism, contemporary research is placing increasing emphasis on its effects on the non-skeletal system. Vitamin D deficiency has been linked to a wide range of diseases, including malignancies, immune system disorders (e.g., Crohn's disease, rheumatoid arthritis), cardiovascular disease, depression and pulmonary dysfunction leading to asthma (15, 18–20). In addition, vitamin D deficiency has been linked to increased susceptibility to infection. In particular, vitamin D plays a role in modulating the immune response, restoring immune homeostasis and reducing organ dysfunction (21). Research indicates that vitamin D can inhibit cellular proliferation and promote the differentiation of various lineages, which is crucial for regenerating epithelial barriers and maturing immune cells. For example, lymphocytes, neutrophils, monocytes, and dendritic cells express the vitamin D receptor (VDR) and act as direct targets of $1,25(OH)_2D_3$. Moreover, these cells facilitate the activation of circulating $25(OH)D_3$ through the hydroxylation activity of the CYP27B1 enzyme (22). The immunoregulatory effects of $1,25(OH)_2D_3$ are evident in its ability to switch between cell-mediated (Th1) and humoral (Th2) immune responses. Additionally, vitamin D enhances macrophage activation and stimulates the synthesis of antimicrobial peptides in epithelial and immune cells, which is vital for clearing bacterial and viral infections.

In critically ill patients, vitamin D serves a significant role as a natural vitamin. The immune response in sepsis functions as a double-edged sword: while an effective immune response is essential for combating infection, an excessive or dysregulated response can result in tissue damage and organ dysfunction. As such, the immunoregulatory function of vitamin D has emerged as a potential therapeutic target. Vitamin D helps maintain immune homeostasis during sepsis and enhances the body's antibacterial capacity by modulating the functions of immune cells, including T lymphocytes, B lymphocytes, and macrophages, regulating cytokine production, and influencing innate immunity (23). For example, T. Greulich et al. elucidated its role in attenuating the inflammatory response and enhancing the antimicrobial activity of innate immune cells, potentially linking deficiency to increased susceptibility to systemic inflammatory response syndrome (SIRS) and sepsis (7). In addition, Prakash Vipul et al. found an inverse association between vitamin D levels and length of hospital stay in septic patients, suggesting an increased risk of mortality in the intensive care setting due to vitamin D deficiency (8). Similarly, research by Megan A et al. highlighted a significant increase in 30-day mortality in septic patients with vitamin D insufficiency (24).

In the context of sepsis, the kidney is particularly vulnerable, with AKI significantly increasing hospitalization rates and mortality risk (25). Vitamin D deficiency in septic patients contributes to AKI through mechanisms that go beyond immune dysfunction. Vitamin D depletion upregulates the renin-angiotensin-aldosterone system (RAAS) and increases the expression of renal angiorenalin mRNA, precipitating AKI (26). In addition, vitamin D insufficiency exacerbates ischemia/reperfusion injury by impairing renal vascular function and accelerates the progression of AKI to chronic kidney injury through modulation of the transforming growth factor-beta-1 signaling pathway, along with reduced expression of the vitamin D receptor (VDR) and Klotho protein (27, 28). Studies by David E. Leaf show a significant inverse association between bioavailable 25(OH)D levels and mortality in AKI patients, adjusting for age and blood creatinine (29). Lingyun Lai's research also shows that 1,25-dihydroxyvitamin D levels decrease with increasing severity of AKI. In addition, low vitamin D levels are identified as a risk factor for AKI and are associated with a poorer prognosis once AKI manifests (30). Lynda K. Cameron et al. suggest that critically ill patients with moderate to severe AKI have significantly lower serum $1,25(OH)_2D$ concentrations than those without AKI, and that recovery from AKI correlates with increased serum $1,25(OH)_2D$ levels. Early assessment of vitamin D status and supplementation may therefore attenuate the progression of kidney disease and improve patient outcomes (31).

Despite numerous studies investigating the utility of vitamin D as a therapeutic intervention for SA-AKI, there is ongoing debate about its efficacy in improving patient outcomes. Evidence from studies investigating vitamin D supplementation during hospitalization has demonstrated a significant reduction in in-hospital mortality in patients with chronic obstructive pulmonary disease (COPD), suggesting potential benefits that extend to patients with sepsis (32, 33). For example, Tzu-Hsien Liao et al. have proposed that appropriate vitamin D supplementation may attenuate the progression and severity of AKI in animal models, although translation to humans remains to be validated (34). Preclinical studies have also highlighted the immunomodulatory effects of vitamin D in attenuating lipopolysaccharide-induced oxidative stress and renal expression of inflammatory cytokines, particularly relevant in SA-AKI (28, 35). The present study uses data from the

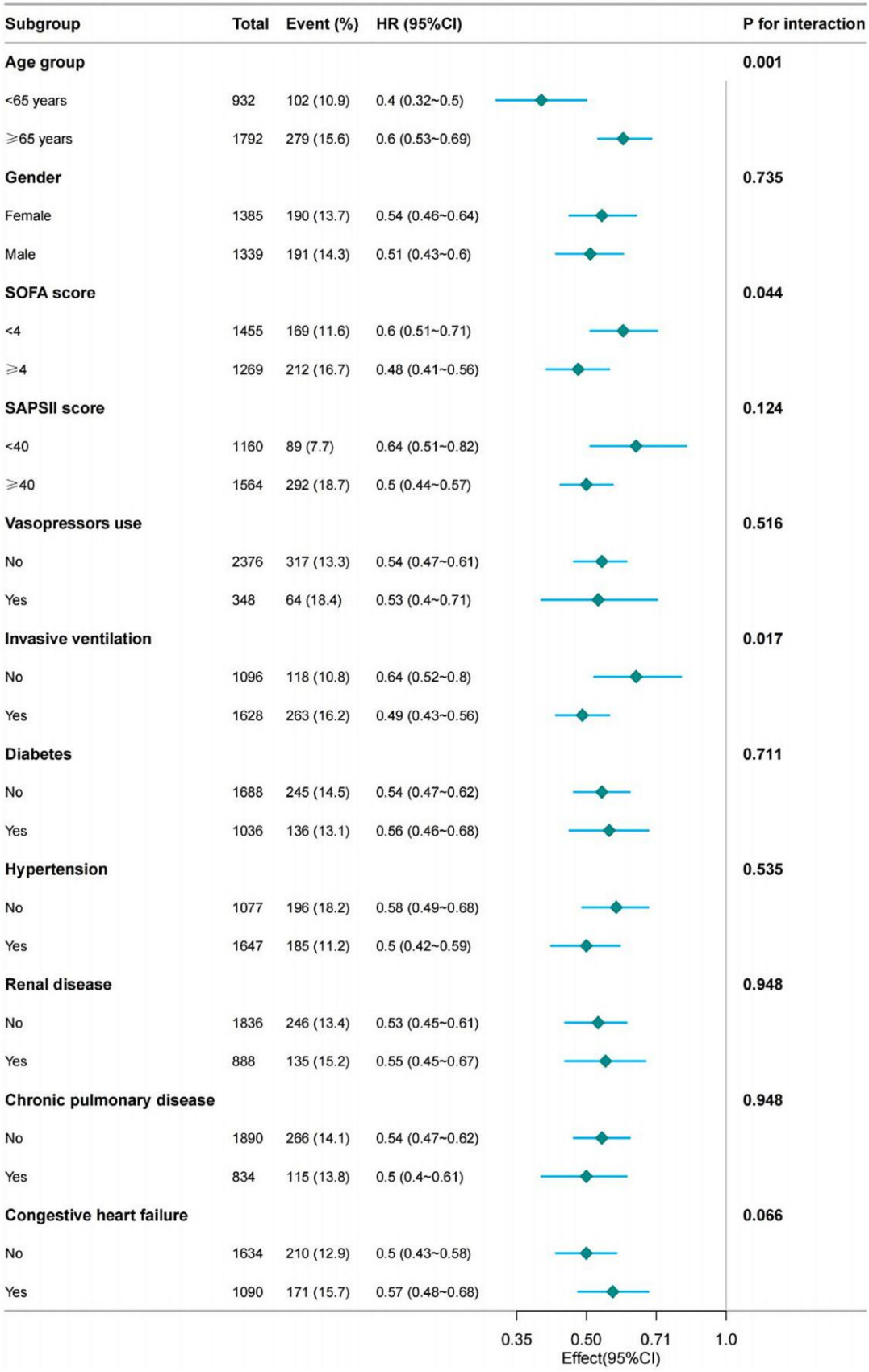

FIGURE 4
Forest plot shows HRs of in-hospital mortality in vitamin D group in subgroup analyses.

extensive MIMIC database to analyze the association between vitamin D supplementation on admission and prognosis in critically ill SA-AKI patients, showing a reduced risk of mortality with early vitamin D administration. Given the high prevalence of vitamin D deficiency in critically ill populations, particularly those with underlying chronic kidney disease, adjustments for confounding variables were carefully applied in subgroup analyses to ensure robust results. These findings highlight the potential of timely and appropriate vitamin D supplementation not only to modulate immune responses and enhance antimicrobial defenses, but also to attenuate the progression of ischemia-reperfusion kidney injury and improve patient prognosis.

This study is the first to examine the relationship between vitamin D supplementation and prognosis in SA-AKI patients admitted to intensive care. Rigorous statistical methods were applied to ensure robust results, taking advantage of the large sample size from the MIMIC-IV database. However, several limitations should be noted. The retrospective design precluded access to baseline and post-treatment vitamin D levels, which may have limited the findings. Despite efforts to control for confounding variables, residual confounding may still exist. In addition, the study focused exclusively on the binary presence of vitamin D supplementation, without exploring optimal dosing or administration protocols. Future prospective studies are needed to clarify the most effective strategies for vitamin D supplementation in this patient population.

5 Conclusion

Vitamin D supplementation has been demonstrated to reduce in-hospital mortality, as well as 28-day and 90-day mortality, in patients with SA-AKI in the ICU, while also increasing the number of days without norepinephrine administration within the 28-day period. This cost-effective and safe intervention involves testing vitamin D levels in critically ill patients and initiating supplementation promptly, potentially improving patient outcomes. However, further clinical trials are needed to provide definitive evidence of its efficacy in improving the prognosis of SA-AKI patients hospitalized in ICUs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Institutional Review Board of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center for the studies involving humans because the studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this is a publicly available anonymized database. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because this is a publicly available anonymized database.

Author contributions

JS: Data curation, Methodology, Writing – original draft, Writing – review & editing. YW: Writing – original draft, Writing – review & editing, Supervision, Validation. JW: Supervision, Validation, Writing – original draft, Writing – review & editing. HW: Writing – original draft, Writing – review & editing, Data curation. ZX: Data curation, Writing – original draft, Writing – review & editing, Validation. DN: Validation, Writing – original draft, Writing – review & editing, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association between 25(OH) vitamin D and schizophrenia: shared genetic correlation, pleiotropy, and causality

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Background: This study delves into the complex interplay between genetics, 25-hydroxyvitamin D (25OHD), and schizophrenia (SCZ). It leverages extensive sample data derived from Genome-Wide Association Studies (GWAS) to uncover genetic correlations.

Methods: Employing Linkage Disequilibrium Score Regression (LDSC) and S-LDSC, this study investigates genetic connections between 25OHD and SCZ. It examines Single Nucleotide Polymorphism (SNP) heritability in specific tissues and incorporates diverse immune cell datasets for genetic enrichment analysis. Local genetic correlations were analyzed using HESS software, and pleiotropy analysis identified shared genetic loci in brain tissues. Hyprcoloc analysis was used to explore shared genetic factors between 25OHD, immune cells, and SCZ, complemented by a bidirectional Mendelian Randomization (MR) to probe potential causal links.

Results: We identified a significant negative genetic correlation between 25OHD levels and SCZ. PLACO analysis revealed 35 pleiotropic loci with strong enrichment in brain regions, particularly the cerebellum, frontal cortex, and hippocampus. Eight loci (1p34.2, 2p23.3, 3p21.1, 5q31.2, 12q23.2, 14q32.33, 16p13.3, and 16q24.3) exhibited strong colocalization, highlighting potential drug targets. Gene and tissue enrichment analyses emphasized neurological and immune-related mechanisms, including hyaluronan metabolism. Bidirectional MR analysis supported a causal effect of SCZ on 25OHD levels.

Conclusion: Our study identifies NEK4 as a potential therapeutic target and highlights the involvement of hyaluronan metabolism in the genetic association between 25OHD and SCZ. These findings provide valuable insights into shared genetic pathways, immune-related connections, and causal interactions in the context of SCZ.

KEYWORDS

vitamin D, schizophrenia, genetic overlap, Mendelian randomization, genome-wide association study

1 Introduction

Schizophrenia (SCZ) is a complex mental disorder that affects about 21 million people worldwide (1). Although the pathogenesis of SCZ is unclear, SCZ has been shown to have etiological links to events early in life, at birth and even *in utero* (2, 3). Epidemiological evidence has demonstrated that a person's risk of developing SCZ is influenced by factors such as childhood trauma, environmental displacement, social isolation, advancing urbanization, and substance abuse (4). In more detail, the prevalence is higher in children born in winter, living at high latitudes and growing up in cities (5–7). All of these factors are associated with reduced sunlight exposure, so low 25OHD levels are considered a risk factor for SCZ (8).

Most vitamin D is synthesized in the skin with the help of sunlight, and only a small portion is taken in through the diet (9). The amount of vitamin D synthesized depends on age, skin color, season, duration of sun exposure, and latitude of residence (10). Vitamin D3 is first hydroxylated in the liver to produce 25OHD, and then the physiologically active 1, 25 dihydroxyvitamin 2D (1, 25OH2D) is produced in the kidneys, so serum 25OHD levels are the best parameter to represent vitamin D levels (11). Recent studies have found that 25OHD, in addition to its involvement in the regulation of calcium and phosphorus metabolism in the body and the maintenance of bone health, is potentially linked to psychiatric disorders such as SCZ, depression and anxiety (12, 13). The association may be predicated on the fact that both 25OHD and 1, 25OH2D can cross the blood-brain barrier, and that vitamin D receptors and vitamin D metabolizing enzymes are present in human and rodent brain tissue (14–17). 25OHD increases the number of mature macrophages, raises the expression levels of CD36 and PPAR-γ in the brain, benefits neurological recovery and promotes the clearance of blood masses in mice after cerebral hemorrhage (18). Imaging studies have found a significant positive correlation between 25OHD levels and hippocampal volume in patients with SCZ (19). 25OHD has an important protective effect on the hippocampus.

Low levels of 25OHD in fetuses or newborns increase risk of SCZ (20). A Danish case-control study (N = 868) found a significant association between low 25OHD levels in newborns and the risk of SCZ later in life (21). Another case-control study (N = 2,602) with a larger sample size replicated the association of neonatal 25OHD deficiency with a significantly increased risk of SCZ (22). Such evidence points to the hypothesis that maternal 25OHD deficiency is a risk factor for SCZ (8). The meta-analysis found that people with SCZ were more likely to be 25OHD deficient (23, 24). However, this does not confirm an independent association: people with SCZ generally have reduced exercise and diet and do not spend enough time in the sun, which are known to contribute to lower 25OHD levels. In conclusion, controversy with regard to the true link between 25OHD and SCZ has been unsettled.

Traditional observational epidemiologic studies have many limitations in establishing causal relationships between disease exposures and outcomes, including reverse causal associations with potential confounders that bias results and render them unreliable (25). Well-designed randomized controlled trials are the gold standard for determining the association between 25OHD levels and the risk of SCZ, but are difficult to conduct for large samples followed over decades. By integrating genetic epidemiological methods such as Mendelian randomization (MR), pleiotropy, and genetic correlation analyses, potential associations in complex phenotypes can be explored more reliably. This approach has demonstrated its advantages in research across multiple fields (26–28). Numerous studies have shown that 25OHD is involved in evolutionary processes associated with SCZ pathogenesis, and genetic variation may influence such neurobiological processes directly or indirectly through 25OHD levels. Therefore, it is important to further investigate potential overlapping genetic structures. Based on these factors, we systematically evaluated the complex relationship between 25OHD and SCZ in terms of genetic correlation, pleiotropy, and causality (Figure 1).

2 Materials and methods

2.1 Study design

In order to assess the complex genetic relationship between 25OHD and SCZ, this present study evaluated the genetic correlation between them using the linkage disequilibrium score regression (LDSC) method, and identified the corresponding pleiotropic loci and genes between the two phenotypes using the composite null hypothesis (PLACO). Finally we assessed the causal associations between 25OHD and SCZ using two-sample bidirectional MR method.

2.2 25OHD data

25OHD data were obtained from a genome-wide association study (GWAS) of 25OHD levels in 401,460 white British samples (29). The study measured 25OHD levels in twice-collected blood samples in a linear mixed model GWAS of standardized log-transformed 25OHD levels with covariates including age, sex, season, and vitamin D supplementation. A meta-analysis of GWAS results from 42,274 European samples was also performed, resulting in the detection of 138 conditionally independent Single Nucleotide Polymorphisms (SNPs, 63 novel) and an estimated SNP heritability of 16.1% for the 25OHD.

Abbreviations: 25OHD, 25-hydroxyvitamin D; SCZ, schizophrenia; CNS, central nervous system; PLACO, pleiotropic locus colocalization analysis; MR, Mendelian randomization; LDSC, linkage disequilibrium score regression; HDL, high-definition likelihood; HESS, heritability estimation from summary statistics; SNP, single nucleotide polymorphism; eQTL, expression quantitative trait loci; S-LDSC, stratified linkage disequilibrium score regression; cDC, conventional dendritic cells; TBNK, T cells, B cells, natural killer cells; MFI, median fluorescence intensity; BA9, Brodmann Area 9; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; QQ, quantile-quantile; GWAS, genome-wide association study; IVs, instrumental variables; FDR, false discovery rate; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAGMA, Multi-marker Analysis of Genomic Annotation; ILC3, group 3 innate lymphoid cells; NKp46, natural killer cell p46-related protein; ICD, International Classification of Diseases; NEK4, NIMA-related kinase 4; MSigDB, Molecular Signatures Database; PP, posterior probability; MDD, major depressive disorder; BIP, bipolar disorder; CVD, cardiovascular disease.

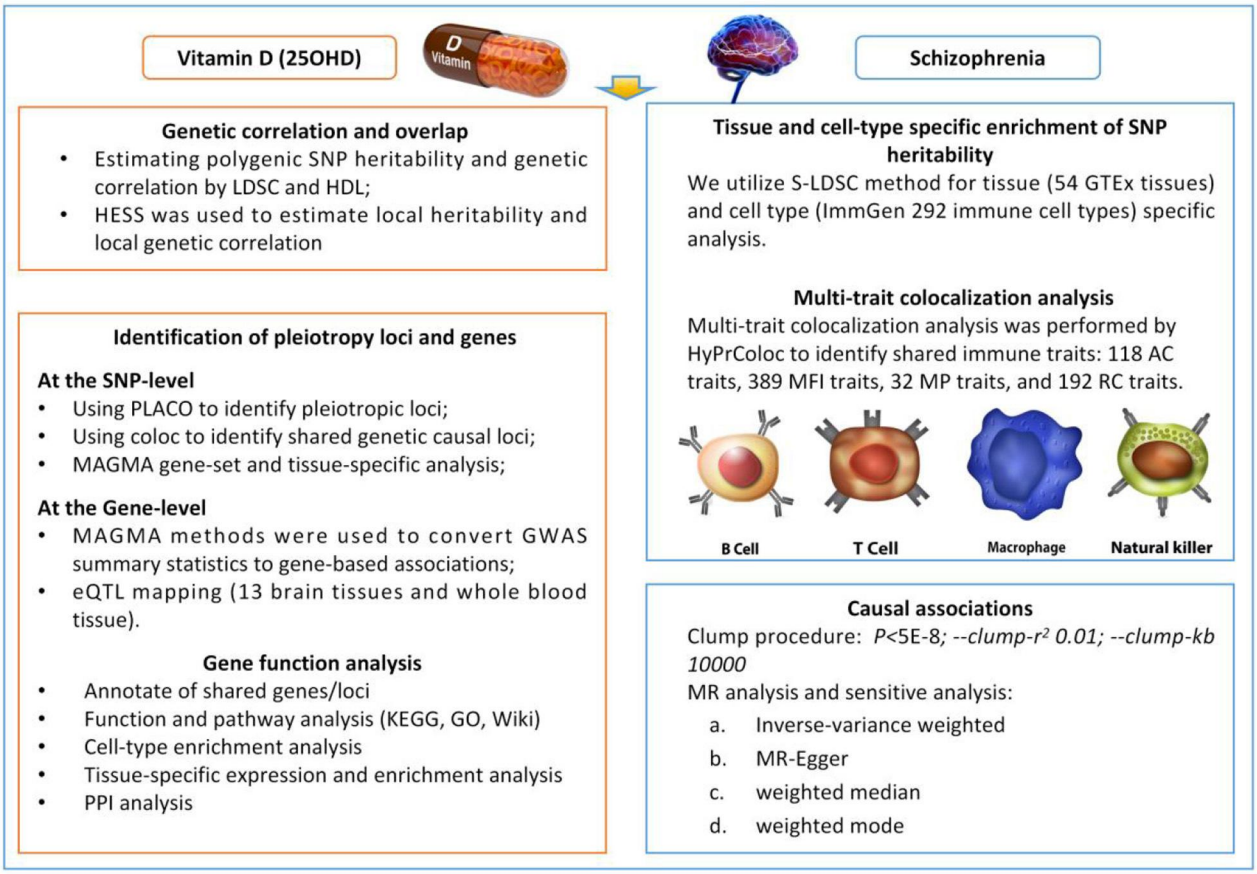

FIGURE 1
Overview of statistical analyses performed in the study. SNP, Single Nucleotide Polymorphism; LDSC, Linkage Disequilibrium Score Regression; HDL, high-definition likelihood; HESS, Heritability Estimation from Summary Statistics; PLACO, Protein–Ligand Affinity by Comparable Co-Evolution; MAGMA, Multi-marker Analysis of GenoMic Annotation; GWAS, Genome-Wide Association Study; eQTL, Expression Quantitative Trait Locus; S-LDS, Stratified Linkage Disequilibrium Score.

2.3 Schizophrenia data

The GWAS summary data for SCZ were derived from a two-stage genome-wide association study involving 55,365 European SCZ patients and 219,884 control individuals (30). This study reported 287 common variants associated with SCZ. In our study, we selected only European ancestry samples to align with the 25OHD dataset. In each individual cohort, association tests were performed using an additive logistic regression model with PLINK, and the results were finally meta-analyzed using an inverse variance-weighted fixed-effects model.

2.4 Linkage disequilibrium score regression

The Linkage Disequilibrium Score Regression (LDSC) method was used to analyze genetic correlations, showing how different traits might share common genetic factors (31). This method calculates the extent of shared genetic structures between traits, which helps identify their potential genetic links. LD scores in LDSC were calculated using European ancestry samples from the 1,000 Genomes Project and the HapMap3 project as reference panels (32). Additionally, high-definition likelihood (HDL) analysis was performed to increase the precision of these estimates and enhance statistical power (33), using a reference panel from approximately 300,000 individuals with European ancestry in the UK Biobank. For SNPs, stringent quality control measures were implemented: (1) Exclusion of non-biallelic SNPs and SNPs with ambiguous strand information; (2) Exclusion of SNPs without rs tags; (3) Removal of duplicate SNPs or those not present in the 1,000 Genomes Project or with mismatched alleles; (4) SNPs located within the major histocompatibility complex (chr6: 28.5–33.5 Mb) region were excluded from LDSC analysis due to their complex LD structure. (5) SNPs with a minor allele frequency (MAF) >0.01 were retained. Missing data were handled by excluding SNPs with incomplete information to prevent potential biases in the analysis.

2.5 Stratified linkage disequilibrium score regression

We used Stratified Linkage Disequilibrium Score Regression (S-LDSC) to explore whether genetic heritability for 25OHD and SCZ

is enriched in specific cell types and tissues, such as the brain or immune cells. This method helps determine if certain tissues or cell types show stronger genetic influences related to these traits, providing insights into where these genetic effects are most active. We obtained human tissue data from GTEx consortium, including 54 different tissues (comprising 13 distinct brain tissues) (34), and obtained data on 292 immune cell types from the ImmGen consortium (35). After adjusting for baseline models and all gene sets, we used the *p*-values of the regression coefficient z-scores to evaluate the significance of SNP heritability enrichment estimates in each tissue and immune cell type.

2.6 Local genetic correlation analysis

We applied the Heritability Estimation from Summary Statistics (HESS) to estimate local genetic correlation and assessed the genetic overlap in distinct phenotypes within locally independent genomic regions (36). This approach helps identify areas in the genome where genetic overlap between 25OHD and SCZ may be concentrated, providing more detailed insights into specific genetic regions. A total of 1,702 independent LD regions were included in the analysis. In the first step, HESS computed the eigenvalues of the LD matrix, as well as the squared projection of the GWAS effect size vectors onto the eigenvectors of the LD matrix. In the second step, HESS utilized the output from step 1 to estimate local SNP heritability and its standard error. In the third step, HESS used the output from step 2 to obtain estimates of local genetic covariance and their standard errors. The reference dataset was constructed based on the hg19 genome using samples from the 1,000 Genomes European population.

2.7 Pleiotropic analysis under composite null hypothesis

SNP-Level PLACO is a novel method used to study the polygenic loci underlying complex traits using only summary-level genotype-phenotype association statistics (28, 37, 38). The purpose of PLACO analysis is to identify genetic regions that may be shared between 25OHD and SCZ. This type of pleiotropy analysis helps us pinpoint genetic loci that are linked to both traits, revealing potential shared genetic foundations and biological mechanisms. Specifically, we computed the square of the Z-scores for each variant and removed SNPs with extremely high Z^2 (>80). Considering the potential correlation between 25OHD and SCZ, we estimated the correlation matrix of Z-scores. Subsequently, we used a level-α intersection-union test (IUT) method to assess the hypothesis of no pleiotropy. The final *p*-value for the IUT test was determined by the maximum *p*-value between H_0 and H_1.

Based on the PLACO results, we further mapped the identified loci to nearby genes to explore the shared biological mechanisms of these polygenic sites. We conducted a Generalized Gene-Set Analysis of GWAS Data (MAGMA) analysis on genes located at or overlapping with the polygenic loci identified based on PLACO output and single-trait GWAS to identify candidate pathways associated with polygenicity and tissue enrichment of polygenic genes (39). Functional Mapping and Annotation (FUMA) using genome-wide association study data were utilized to determine the biological functions of the polygenic loci (40). Pathway enrichment analysis was performed based on a range of pathways using the Molecular Signatures Database (MSigDB) to determine the functions of the mapped genes (41). eQTL analysis incorporated SNP-gene association data from whole blood tissues.

2.8 Colocalization analysis

To identify whether shared genetic regions between 25OHD and SCZ are likely due to common causal variants, we conducted Bayesian colocalization analysis using the R package "coloc." This method helps determine if two traits have a true shared genetic cause at a given locus or if they simply overlap by chance. Colocalization analysis relies on the assumption of a single causal variant, and for each multi-trait locus, it yields posterior probabilities (PP) for five hypotheses: H_0: Neither of the two traits has a genetic association in this region; H_1: Only trait 1 has a genetic association in this region; H_2: Only trait 2 has a genetic association in this region; H_3: Both traits are associated but have different causal variants; H_4: Both traits are associated and share a common causal variant. The "coloc.abf" function was employed for colocalization analysis ($P_1 = P_2 = 1 \times 10^{-4}$, $P_{12} = 1 \times 10^{-5}$).

2.9 Hypothesis prioritization for multi-trait colocalization analysis

We utilized the Hypothesis Prioritization for Multi-Trait Colocalization (HyPrColoc) method (42) to conduct multi-trait colocalization analysis by integrating GWAS summary statistics for 731 immune cells from the GWAS catalog (ranging from GCST0001391 to GCST0002121) (43). This analysis aimed to underscore the pivotal role of immune cells in the progression of 25OHD and SCZ. The immune cell traits encompassed various aspects, including 118 absolute cell counts (AC) traits, 389 median fluorescence intensity (MFI) reflecting surface antigen levels traits, 32 morphological parameters (MP) traits, and 192 relative cell counts (RC) traits. Notably, MFI, AC, and RC features covered a range of immune cell subsets, such as B cells, CDC, Ts cell maturation stages, mononuclear cells, bone marrow cells, TBNK, and Treg panels. The MP features were specific to CDC and TBNK panels. Primary GWAS analyses for 731 Immune traits were conducted on a cohort of 3,757 individuals of European descent, with covariates including sex, age, and age^2. Furthermore, a reference panel based on European sequences was leveraged to estimate approximately 22 million SNPs genotyped using high-density arrays.

2.10 Mendelian randomization analysis

MR analysis in this study aims to investigate whether there is a potential causal relationship between 25OHD and SCZ. By using genetic variants as instrumental variables (IVs), MR helps estimate

causal effects in a way that minimizes bias from other confounding factors. In this study, significant genetic loci are carefully selected, and sensitivity analyses ensure the reliability of the results, even when different assumptions are tested. This approach allows us to infer causality rather than just correlation, making it a powerful tool for understanding complex relationships in genetic data. In our analysis, we employed the clumping procedure in the PLINK software to select independently significant genetic loci as instrumental variables for two traits ($P < 5 \times 10^{-8}$) (41). The r^2 threshold for instrumental variables was set at 0.001, with a physical distance of 10,000 kb window. We also calculated the r^2 and F-statistic for each of them to ensure the strength of the instrumental variables (44) and the formula for calculating the F-statistic is as follows:

$$F = (\frac{n-1-k}{k})(\frac{r^2}{1-r^2})$$

Here, r^2 represents the proportion of variance explained by the instrumental variable, n is the sample size, and k is the number of SNPs.

The primary method used for Mendelian randomization (MR) was the Inverse Variance Weighted (IVW) method, which requires IVs to satisfy three assumptions: (1) IVs should be associated with the exposure; (2) IVs should not be associated with confounding factors related to both the exposure and the outcome; (3) The effect of IVs on the outcome is entirely mediated through the exposure. Several sensitivity analyses were conducted. First, the heterogeneity Q test of IVW and MR-Egger was used to detect potential violations of the assumptions through heterogeneity among the individual IVs (45). Second, intercept of MR-Egger was applied to estimate the horizontal pleiotropy, ensuring that genetic variation is independently associated with exposure and outcome (46). Additional analyses using different modeling assumptions and robust MR methods (weighted median and weighted mode) were employed to enhance the stability and robustness of the results.

All statistical analyses were performed using R version 3.5.3 software, and Mendelian randomization analyses utilized the Mendelian Randomization package (47).

3 Results

3.1 Genetic correlation between 25OHD and SCZ

In this study, we observed a significant genetic correlation between 25OHD and SCZ ($rg = -0.083$, $P = 1 \times 10^{-4}$) by LDSC. The intercept was −0.008 (with a standard error of 0.007), excluding the possibility of sample overlap between 25OHD and SCZ data. The LDSC method without constraining the intercept yielded consistent results ($rg = -0.091$, $P = 3.48 \times 10^{-9}$). Additionally, the HDL method also provided nearly identical results ($rg = -0.103$, $P = 2.96 \times 10^{-4}$). Supplementary Table S1 provides a detailed breakdown of the genetic correlations between 25OHD and SCZ, showing the consistency of results across different methods such as LDSC and HDL. The table highlights the significant negative genetic correlation observed and further supports the robustness of these findings. By using HESS, we estimated significant local genetic correlations in some genomic regions and observed negative genetic overlaps in these overlapping regions. But no significant difference was observed in respective local genetic heritability estimated for SCZ and 25OHD (Figure 2 and Supplementary Table S2).

3.2 Pleiotropic GeneLoci identified for 25OHD and SCZ

By using PLACO analysis, we identified a total of 35 independent pleiotropic loci between 25OHD and SCZ, with eight genomic regions (FOXO6, GCKR, NEK4, CTB-35F21.1, RP11-328J6.1, PPP1R13B, CDIP1, and FANCA) having PP.H4 values greater than 0.7, indicating their significance in the association between 25OHD and SCZ. Figure 3A is the Manhattan plot of the identified signals, and detailed information on the identified pleiotropic loci was presented in Table 1 and Supplementary Table S3, including the genomic positions and their associated genes. We didn't find any evidence of genome inflation in the QQ Plot (Supplementary Figure S1), indicating that the results are free from systematic biases. Additionally, Supplementary Figure S2 provides essential information on each genomic risk locus, offering a detailed overview of the genetic architecture and variability across loci in the study. Supplementary Figure S3 shows significant enrichment of pleiotropic SNPs in intronic and intergenic regions, particularly in introns, suggesting a key role for non-coding regions in the genetic overlap between 25OHD and SCZ. Other regions, such as downstream and exonic areas, also show some enrichment but to a lesser extent. Regional plots for each risk locus are presented in Supplementary Figures S4–S11. Gene set enrichment analysis using MAGMA was performed based on the results of pleiotropy (Table 2), and the top 3 pathways were statins inhibit cholesterol production, plasma lipoprotein remodeling in responder group and vitamin D Metabolism. Furthermore, we identified nominal significant enrichment in the top six tissues ($P < 1 \times 10^{-3}$) using MAGMA tissue-specific analysis (Figure 4A), with five of these significantly enriched tissues being of cerebral origin, including the cerebellar hemisphere, cerebellum, and frontal cortex (BA9). These brain regions exhibited the highest enrichment levels, underscoring their potential involvement in the genetic relationship between 25OHD and SCZ (Supplementary Table S4). Additionally, the pituitary gland was also among the significantly enriched tissues, further highlighting the role of brain-related tissues in this association. It is worth noting that this section of the MAGMA gene set and tissue-specific analysis was conducted using the complete distribution of SNP *p*-values.

3.3 Identification of priority pleiotropic genes and functional enrichment

The pleiotropic genes were mapped using three different approaches, including the mapping of nearby genes based on the physical position of lead SNPs, MAGMA gene-level

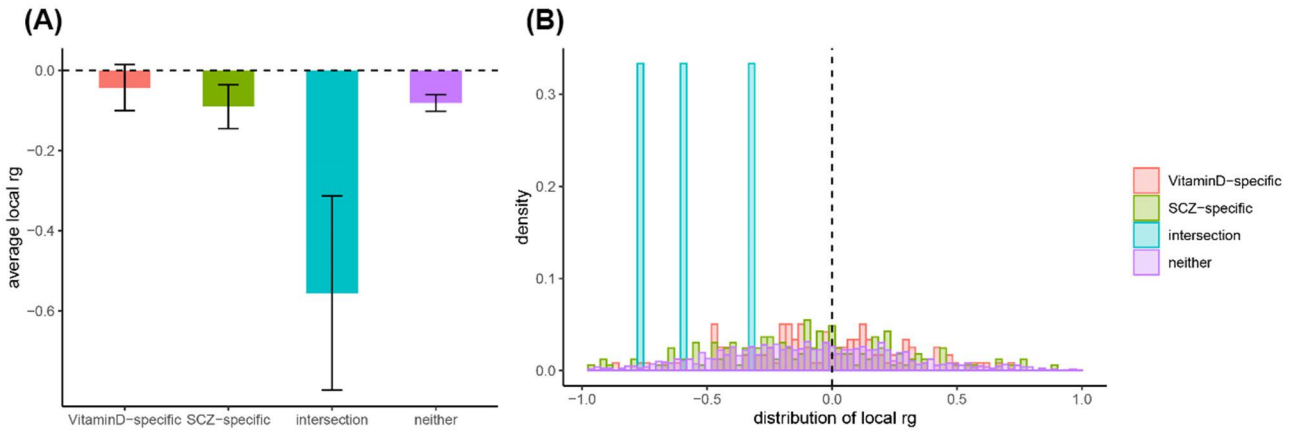

FIGURE 2
Analysis of local genetic correlation and heritability distribution across different genomic regions. **(A)** Average local genetic correlations (rg) for Vitamin D-specific, SCZ-specific, and interaction regions, highlighting the significant negative correlation in the interaction regions. **(B)** Frequency distribution of local genetic correlations, showing a concentration of negative correlations in interaction regions, suggesting a complex negative genetic relationship between Vitamin D and SCZ in these areas.

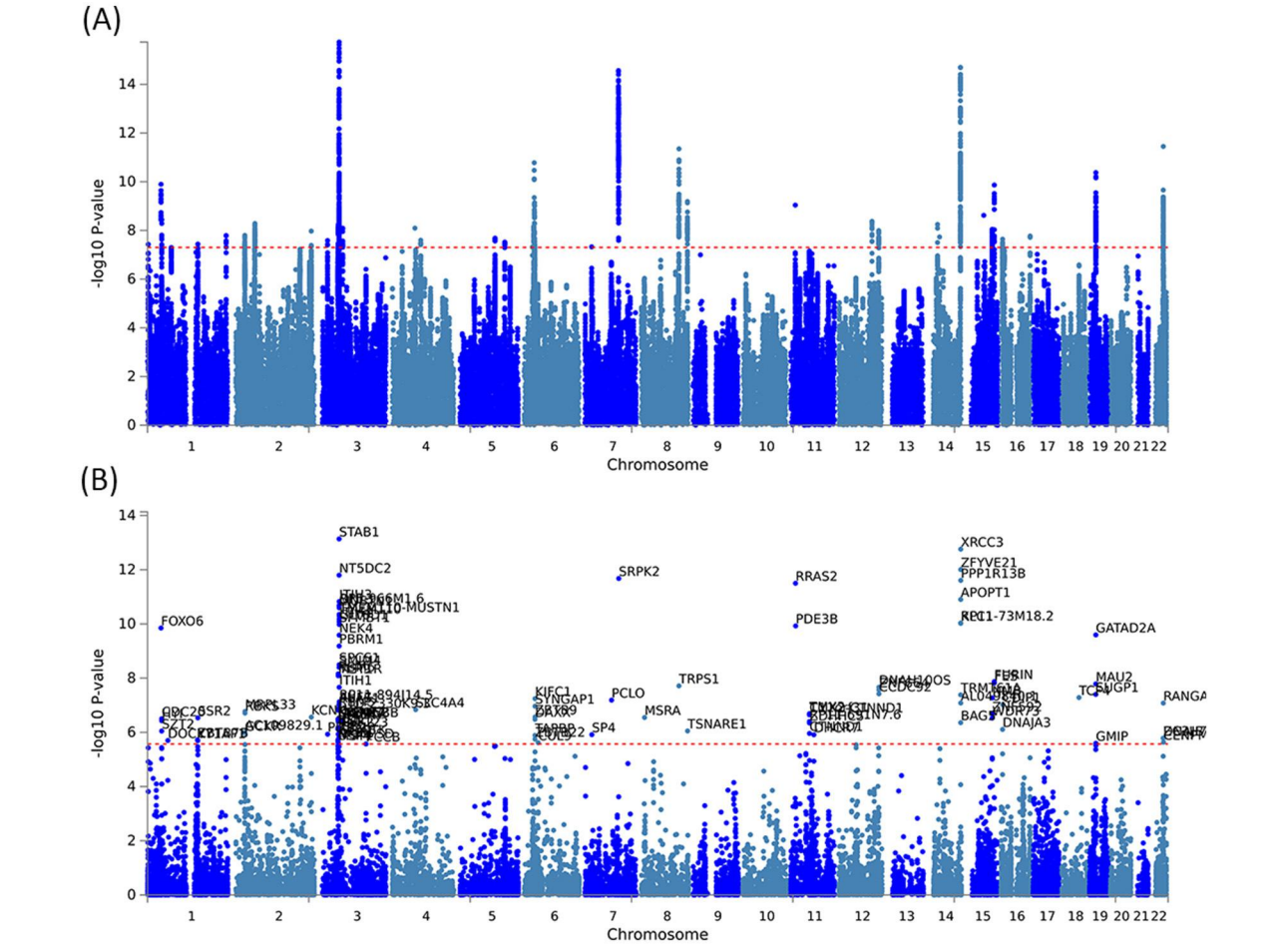

FIGURE 3
Manhattan plots illustrating pleiotropic associations between 25OHD and SCZ. **(A)** Manhattan plot showing pleiotropic SNP signals between 25OHD and SCZ, with significant associations observed on chromosomes 3, 7, 8, 14, and 19. **(B)** Manhattan plot highlighting pleiotropic genes identified by the MAGMA method, such as STAB1 and NT5DC2, which are significantly associated with both 25OHD and SCZ.

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TABLE 1 Information on 35 pleiotropic loci identified between 25OHD and SCZ.

Genomic locus	Locus region	Lead SNP	P	P_{adj}	Symbol	PP.H4
1p36.32	1:2274325-2631294	rs6688934	3.74E-08	0.037	PLCH2	0.024
1p34.2	1:41467273-41933422	rs10218712	1.28E-10	<0.001	FOXO6	0.986
1p34.2	1:43760070-44484269	rs11210887	3.63E-09	0.004	PTPRF	0.014
1q22	1:155043400-156081240	rs12043212	3.65E-08	0.037	RP11-336K24.5	0.606
1q43	1:243019418-244110411	rs12743378	1.63E-08	0.016	SDCCAG8	0.661
2p23.3	2:27284987-28503941	rs6744393	1.60E-08	0.016	GCKR, AC109829.1	0.718
2p16.1	2:58132104-59120088	rs2215966	5.22E-09	0.005	LINC01122	0.581
2q37.1	2:233550713-233821491	rs6704768	1.07E-08	0.011	GIGYF2	0.017
3p24.3	3:16687078-17151806	rs9876137	2.61E-08	0.026	PLCL2	0.037
3p21.31	3:49044713-50593986	rs6765484	5.81E-09	0.006	RBM6	0.676
3p21.1	3:52214640-53539241	rs7646741	1.84E-16	<0.001	NEK4	0.840
3p14.1	3:63674722-64107217	rs704364	8.14E-09	0.008	ATXN7	0.201
4q13.2	4:69497139-70517902	rs41297381	8.06E-09	0.008	UGT2B4	0.343
4q22.1	4:87983900-88430396	rs28445336	2.53E-08	0.025	HSD17B11	0.444
5q21.3	5:108642367-109222729	rs253245	2.08E-08	0.021	KRT18P42, AC012603.1	0.515
5q31.2	5:138346820-139391816	rs4912756	3.04E-08	0.030	CTB-35F21.1	0.738
6p22.2	6:25147518-27124904	rs198811	4.83E-08	0.048	HIST1H2AC	0.040
6p21.32	6:33191338-33864288	rs10807124	3.89E-08	0.039	SYNGAP1	0.388
7p15.3	7:21285713-21713646	rs6461561	4.73E-08	0.047	SP4	0.377
7q22.3	7:104410300-105096449	rs2428162	2.74E-15	<0.001	LINC01004, RP11-325F22.2	0.151
8q23.3	8:116481788-117135574	rs1109537	4.47E-12	<0.001	LINC00536	0.326
8q24.3	8:143250850-143534403	rs13262595	6.51E-10	0.001	TSNARE1	0.005
11p15.2	11:13674659-14268378	rs11023064	9.17E-10	0.001	SPON1	0.316
12q23.2	12:103261749-103906212	rs10860964	4.24E-09	0.004	RP11-328J6.1, C12orf42	0.947
12q24.31	12:124149975-124597359	rs79478560	1.02E-08	0.010	DNAH10	0.649
14q13.1	14:33257822-33359973	rs12883788	5.69E-09	0.006	AKAP6, NPAS3	0.061
14q21.1	14:38783868-40213851	rs8011075	1.86E-08	0.019	SEC23A	0.279
14q32.33	14:103748844-104622780	rs4906378	2.00E-15	<0.001	PPP1R13B	0.805
15q21.3	15:58718230-58785679	rs12914626	2.41E-09	0.002	RP11-355N15.1	0.586
15q25.2	15:84263509-85747441	rs62019457	9.06E-09	0.009	UBE2Q2P1	0.029
15q26.1	15:91390454-91481048	rs6224	1.37E-10	<0.001	FURIN	0.312
16p13.3	16:4375753-4811179	rs2058811	2.30E-08	0.023	CDIP1	0.966
16q24.3	16:88963011-90239983	rs78004870	1.71E-08	0.017	FANCA	0.987
19p13.11	19:19068718-20159711	rs2965185	4.23E-11	<0.001	GATAD2A	0.000
22q13.2	22:40818166-42577604	rs9607782	3.57E-12	<0.001	RP1-85F18.5	0.214

Lead SNP was the SNP with minimum *P* values within the corresponding locus. PP.H4 was the posterior probability of H4 calculated by coloc analysis; the Locus boundary was defined as "chromosome: start-end." PP.H4, the posterior probability of H4; 25OHD, 25-hydroxy vitamin D; SCZ, schizophrenia.

associations, and eQTL analysis (Supplementary Table S5). Supplementary Figure S12 and Supplementary Table S6 provide a comprehensive heatmap of gene expression levels across various tissues, with notable expression observed in the brain cortex, hippocampus, and cerebellum. Supplementary Figure S13 and Supplementary Table S7 illustrate the enrichment patterns of

TABLE 2 MAGMA gene-set analysis results (top 10).

Gene set	N genes	Beta	SE	*P*	P_{adj}
WP_STATIN_INHIBITION_OF_CHOLESTEROL_PRODUCTION	29	0.798	0.188	1.14E-05	0.195
REACTOME_PLASMA_LIPOPROTEIN_REMODELING	32	0.728	0.182	3.05E-05	0.518
WP_VITAMIN_D_METABOLISM	9	1.442	0.367	4.33E-05	0.736
NIKOLSKY_BREAST_CANCER_16Q24_AMPLICON	52	0.947	0.242	4.47E-05	0.759
WP_METABOLIC_PATHWAY_OF_LDL_HDL_AND_TG_INCLUDING_DISEASES	17	0.981	0.250	4.52E-05	0.768
GOBP_HIGH_DENSITY_LIPOPROTEIN_PARTICLE_REMODELING	15	1.084	0.278	4.91E-05	0.834
GOCC_POSTSYNAPTIC_SPECIALIZATION	339	0.218	0.056	4.97E-05	0.845
GOBP_REGULATION_OF_FEAR_RESPONSE	8	1.386	0.362	6.32E-05	1.000
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	47	0.603	0.159	7.12E-05	1.000
GOBP_LIPOPROTEIN_BIOSYNTHETIC_PROCESS	94	0.367	0.097	8.23E-05	1.000

25OHD, 25-hydroxy vitamin D; SCZ, schizophrenia; MAGMA, multivariate analysis of genomic annotation. P_{adj} was estimated by Bonferroni method.

gene expression across multiple tissues. Significant enrichment is observed in regions such as the brain cortex, frontal cortex, and hippocampus. These findings suggest that these genes may have important roles in neurological functions, potentially linked to the genetic relationship between 25OHD and SCZ. MAGMA gene analysis identified a total of 105 pleiotropic genes ($P < 2.69 \times 10^{-6} = 0.05/18{,}565$) (Figure 3B and Supplementary Table S8). Supplementary Figure S14 presents the QQ plot, showing a clear deviation from the expected line, particularly in the upper tail. This suggests the presence of significant genetic associations that contribute to the relationship between 25OHD and SCZ. The expression values of these genes across 54 different tissues can be found in Supplementary Figure S15 and Supplementary Table S9. Many genes were observed to be differentially expressed across the 13 brain tissues (e.g., STAB1, GLYCTK, and FOXO6), while several genes (e.g., CDC20, CENPM, and KIFC1) exhibited high expression in EBV-transformed lymphocytes, testis, liver, and whole blood. Tissue-specific enrichment analysis revealed the enrichment of these pleiotropic genes in skeletal muscle and various brain tissues (Supplementary Table S10 and Figure 4B). We further conducted pathway analysis (KEGG, Wiki, GO) after integrating the gene mapping based on positional information and MAGMA gene analysis (Supplementary Figure S16). We observed that these genes were significantly enriched in pathways such as 15q25 copy number variation, presynaptic active zone cytoplasmic component, and hyaluronan metabolic process. Through cell-specific enrichment analysis, the enrichment was primarily observed in three cell phenotypes: "Descartes fetal spleen afp alb positive cells", "Lake adult kidney c3 proximal tubule epithelial cells s1 s2" and "Fan embryonic ctx nsc 2" (Supplementary Figure S17). eQTL mapping analysis was further conducted based on 13 brain and whole blood tissues, identifying a total of 329 pleiotropic genes. For detailed information, please refer to Supplementary Table S5. The overlap of genes between different methods is illustrated in Supplementary Figure S18 and a total of 11 (28%) pleiotropic genes were identified in all three mapping approaches (Supplementary Table S11).

3.4 Immune-related mechanisms shared between 25OHD and SCZ

Through S-LDSC, we observed significant enrichment of SNP heritability in 27 brain tissues and four immune cell phenotypes, which was significantly associated with the pleiotropic results between 25OHD and SCZ ($P < 0.05$) (Figure 5 and Supplementary Table S12). When analyzing the enrichment of immune traits from ImmGen, we also observed two cell phenotypes enriched in the T-cell panel: Tgd.vg2-.Sp and T.4SP24-.Th. Furthermore, within the innate lymphocyte panel, ILC3.NKp46-0.4-.SI, and within the B-cell panel, B.FrE.BM, were identified, suggesting potential shared immune mechanisms.

3.5 Multi-trait colocalization analysis to pinpoint critical immune traits

Our multi-trait colocalization analysis, conducted using the HyPrColoc method, unveiled shared genetic loci among a diverse array of immune cell phenotypes, 25OHD and SCZ. Within the realm of immune cells, we observed significant genetic associations with several traits, such as CD80 on myeloid DCs, CD80 on CD62L+ myeloid DCs, and CD28 on CD39+ activated Treg cells (Supplementary Table S13). Notably, these findings included traits from different panels like cDC, Treg, B cells, and TBNK, which play pivotal roles in the immune system.

3.6 Causal association between 25OHD and SCZ

We employed a bidirectional two-sample MR approach to infer causality between exposure and outcome. The results provided support for a significant causal effect of SCZ on 25OHD levels, but failed to reveal a causal relationship from 25OHD to SCZ (Table 3). We employed both the global

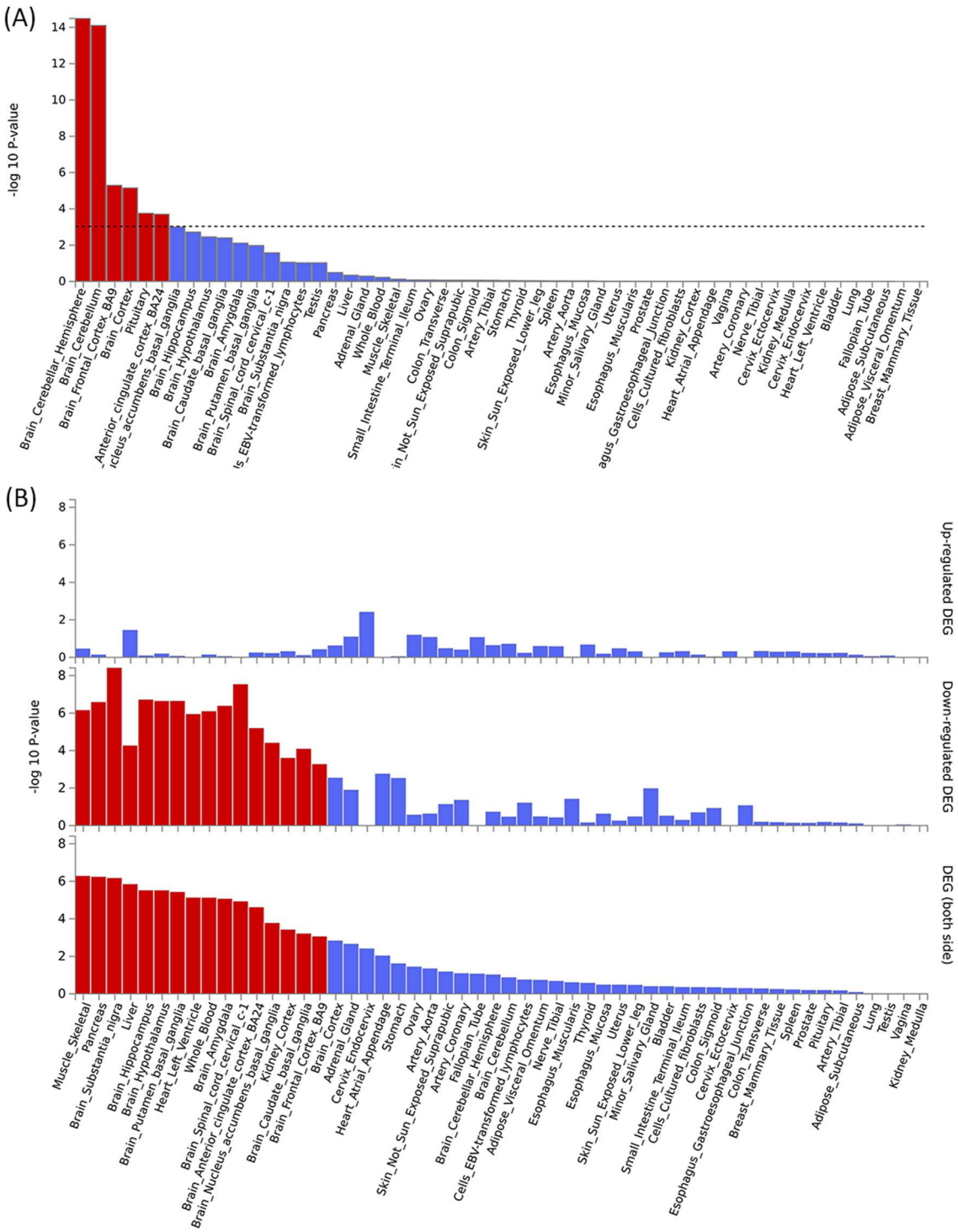

FIGURE 4
Tissue-specific analysis and gene enrichment of pleiotropic signals between 25OHD and SCZ. **(A)** Tissue-specific analysis reveals significant enrichment of pleiotropic signals in brain regions such as the cortex, frontal cortex, and cerebellum. **(B)** The enrichment of pleiotropic MAGMA genes is particularly pronounced in brain tissues, with both upregulated and downregulated genes showing significant associations, highlighting the central role of these regions in the genetic relationship between 25OHD and SCZ.

test of MR-PRESSO and the intercept term of MR-Egger to detect variants with disproportional effects and potential directional pleiotropy. Additionally, we utilized scatter plots and funnel plots to evaluate the stability of the association (Supplementary Figure S19). The scatter plot revealed the absence of multi-effect outliers that could influence causal inference. Meanwhile, the funnel plot displayed a balanced distribution of causal estimates around the effect estimates, confirming the robustness of the results. Supplementary Table S14 presents the information for the IVs.

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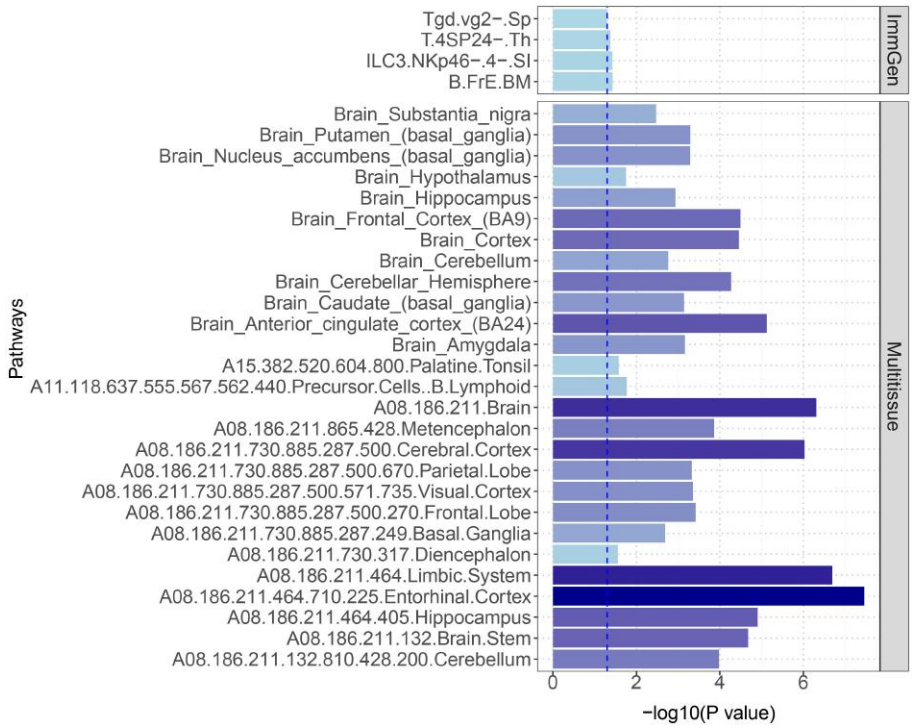

FIGURE 5
S-LDSC estimates of heritability enrichment across different immune cells and brain tissues. The figure highlights significant heritability enrichment in specific immune cells, such as Tgd.vg2-.Sp and T.4SP24-.Th, as well as in key brain regions including the entorhinal cortex, limbic system, and cerebral cortex. These findings suggest critical roles for both immune cells and these specific brain regions in the genetic relationship between 25OHD and SCZ.

TABLE 3 Bidirectional Mendelian randomization analysis results of 25OHD and SCZ.

Exposure	Outcome	Methods	Estimate (95%CI)	*P*	Heterogeneity test	
					Q	*P*
25OHD	SCZ	IVW (fixed)	0.968 (0.904, 1.037)	0.358	171.493	<0.001
		IVW (random)	0.968 (0.889, 1.055)	0.461		
		MR-Egger (slope)	0.95 (0.846, 1.068)	0.389		
		MR-Egger (intercept)	0.001 (−0.003, 0.004)	0.634		
		Weighted mode	0.946 (0.875, 1.024)	0.169		
		Weighted median	0.933 (0.837, 1.04)	0.212		
		DIVW	0.968 (0.886, 1.058)	0.474		
		MR-RAPS	0.949 (0.868, 1.038)	0.254		
SCZ	25OHD	IVW (fixed)	0.983 (0.978, 0.988)	4.35E-10	392.902	<0.001
		IVW (random)	0.983 (0.974, 0.992)	1.58E-04		
		MR-Egger (slope)	0.973 (0.939, 1.008)	0.126		
		MR-Egger (intercept)	0.001 (−0.002, 0.003)	0.554		
		Weighted mode	0.973 (0.955, 0.991)	0.003		
		Weighted median	0.983 (0.974, 0.992)	2.83E-04		
		DIVW	0.983 (0.974, 0.991)	1.40E-04		
		MR-RAPS	0.984 (0.975, 0.993)	5.60E-04		

MR, Mendelian Randomization; IVW, inverse variance weighted; DIVW, debiased IVW; CI, confidence interval; 25OHD, 25-hydroxy vitamin D; SCZ, schizophrenia.

4 Discussion

This study, based on extensive sample data from GWAS research, employed the LDSC method and identified a pronounced genetic correlation between 25OHD and SCZ (rg = −0.083, $P = 1 \times 10^{-4}$). S-LDSC extended this by assessing whether specific tissues, displayed significant enrichment of heritability, helping to localize the genetic influence to biologically relevant tissues. Using HESS, we estimated local genetic correlations, which allowed us to identify specific genomic regions where overlapping genetic effects are concentrated. Pleiotropy analysis then identified pleiotropic loci and found notable gene enrichment in brain tissues, suggesting a potential shared biological basis. To examine immune-related effects, HyPrColoc analysis integrated immune cell data to uncover shared genetic information across 25OHD, immune traits, and SCZ. Finally, bidirectional MR was used to explore potential causal effects between SCZ and 25OHD, allowing us to infer the directionality of their association.

Our study confirmed a significant genetic correlation between 25OHD and SCZ. Bidirectional two-sample MR analysis revealed that SCZ is a risk factor for 25OHD deficiency. Worldwide, the prevalence of 25OHD deficiency is as high as 37.6% in newborns and the elderly (48). A meta-analysis of 25OHD deficiency prevalence revealed that the overall prevalence of 25OHD deficiency in SCZ patients is 65.3%. Individuals with 25OHD deficiency are 2.16 times more likely to develop SCZ compared to those with sufficient levels of 25OHD (24). A more recent meta-analysis found that this prevalence has increased to 70% (23). Two studies based on Danish biobanks have confirmed the association between neonatal 25OHD deficiency and an increased risk of SCZ (21, 22). There is evidence to suggest that genetic variations associated with psychiatric disorders, including major depressive disorder (MDD), bipolar disorder (BIP), and SCZ, are related to lower concentrations of 25OHD. People with risk allele genes associated with psychiatric disorders may have lower 25OHD levels, regardless of clinical diagnosis (49). Most evidence suggests an association between 25OHD deficiency and cardiovascular diseases (CVD) (50). A recent randomized controlled trial involving 21,315 participants found a lower incidence of major cardiovascular events in the group receiving vitamin D3 supplementation (60,000 IU/month) compared to the placebo group, suggesting that vitamin D supplementation may reduce the occurrence of major CVD events (51). Our research findings suggest that individuals with a family history of SCZ should be vigilant about 25OHD deficiency to prevent the later development of CVD events.

Furthermore, a PLACO pleiotropy analysis of both disorders revealed 35 pleiotropic loci, with eight genomic regions (FOXO6, GCKR, NEK4, CTB-35F21.1, RP11-328J6.1, PPP1R13B, CDIP1, and FANCA) having PP.H4 values >0.7, indicating their significance in the association between 25OHD and SCZ. NEK4 has been reported as a shared genetic signal between BIP and SCZ (52). The study identified NEK4 as one of the risk genes associated with neuropsychiatric and substance use disorders. Pathway enrichment analysis revealed an enrichment of XWAS signals in 25OHD gene sets. This suggests that NEK4 may play a significant role in vitamin D metabolic pathways, potentially influencing the risk of disorders such as SCZ. A study identified that alternative splicing of NEK4 is regulated by sQTLs, which are significantly enriched in schizophrenia-associated loci. This suggests that dysregulation of NEK4 splicing may be a key genetic mechanism contributing to SCZ risk (53). A Mendelian randomization study identified a total of 31 promising drug targets for psychiatric disorders, with NEK4 being one of the significant genes specifically associated with SCZ (54). A summary data-based Mendelian randomization (SMR) analysis found that risk alleles in the chromosome 3p21.1 region are associated with NEK4 mRNA expression, and these alleles are significantly linked to SCZ (55). Another study involving 133 first-episode SCZ patients observed a significant association between the severity of SCZ negative symptoms and the risk gene FOXO6, which is associated with BIP (56). A study identifies FOXO6 as a novel candidate gene for SCZ through its pleiotropic effects on educational attainment (EA) and SCZ, revealed by using EA as a proxy phenotype (57). GCKR has been identified as one of the genes associated with alcohol use disorders. Moreover, it exhibits a positive genetic correlation with SCZ, indicating a potential connection between GCKR and SCZ within the framework of alcohol use disorders (58). In summary, among the 35 pleiotropic loci identified, NEK4 shows the strongest evidence and is considered a potential target for the association between 25OHD and SCZ. While there are some studies reporting the relationship between FOXO6 and GCKR with SCZ, there are no reported associations with 25OHD, warranting further investigation.

Tissue-specific analysis revealed significant enrichment of these pleiotropic genes in six tissues, with five originating from brain tissues ($P < 1 \times 10^{-3}$). Our multi-effect results were further subjected to gene analysis using MAGMA, identifying 105 pleiotropic genes. Enrichment analysis demonstrated that these genes are enriched in skeletal muscle and various brain tissues. This emphasizes the potential roles of these gene loci in brain-related processes and their functional impact on skeletal muscle. We further conducted pathway enrichment analysis and found significant enrichment in five pathways: "15q25 copy number variation," "Presynaptic active zone cytoplasmic component," "Hyaluronan metabolic process," "Glyoxylate and dicarboxylate metabolism," and "Oxidoreductase activity, acting on a sulfur group of donors." In a large-scale GWAS meta-analysis investigating the genetic determinants of smoking quantity and their relevance to SCZ, multiple loci were identified. Notably, among these loci, the 15q25 region emerged as a common genetic locus associated with these traits. This locus has been linked to alterations in CHRNA5 expression in the brain (59). Perineuronal nets (PNNs) are highly organized mesh-like networks composed of extracellular matrix molecules surrounding neuronal cell bodies and proximal dendrites. The main components of PNNs include hyaluronan synthase, cartilage link protein-1, and chondroitin sulfate proteoglycans (60). It has been reported that PNN deficiencies can lead to frontal cortex dysfunction in individuals with SCZ, and abnormal PNN formation may increase the risk of SCZ episodes (61). In the context of liver fibrosis induced by sodium arsenite, 25OHD intervention using calcitriol significantly reduces hyaluronic acid levels, suggesting a potential protective effect of vitamin D on liver fibrosis by modulating hyaluronan

metabolism (62). 25OHD deficiency may lead to reduced PNN function, resulting in abnormal cortical gamma band oscillations, thereby increasing the risk of SCZ and worsening the cognitive symptoms of SCZ (63). These observations indicate that the hyaluronan metabolism pathway could be a crucial link between 25OHD and the pathophysiology of SCZ. The synthesis and activation of vitamin D involve multiple enzymes, with some of these enzymes participating in redox reactions involving sulfur atoms within the organism. They alter the structure of vitamin D by introducing oxygen atoms into the molecule, thereby activating it (64). In conclusion, among the five significantly enriched pathways identified, "Hyaluronan metabolic process" shows the strongest evidence, indicating its potential role as a pathway linking 25OHD and SCZ. "15q25 copy number variation" and "Oxidoreductase activity" have some reported associations with SCZ or 25OHD, suggesting they are worthy of further investigation.

25OHD possesses potential immunomodulatory effects (65). Some epidemiological evidence suggests a significant association between 25OHD deficiency and an increased risk or exacerbation of infectious diseases and autoimmune conditions (66–68). A MR study has demonstrated a significant association between various immune cell phenotypes and the risk of SCZ (69). Many immune-related genes and pathways have been shown to be involved in neural development and neuronal function (70). Studies suggest that elevated neutrophil counts and C-reactive protein levels may be more strongly associated with the severity of SCZ (71). Through multi-trait colocalization analysis of GWAS data from 731 immune cell phenotypes, we conducted an in-depth investigation and observed shared genetic loci between multiple immune traits and both 25OHD deficiency and SCZ. We identified 19 immune cell phenotypes with significant genetic overlap (PP $>$ 0.5), including cDC, Treg, B cell, and TBNK panels, among others. These molecules play critical roles in the immune system, deepening our genetic understanding of SCZ and underscoring the importance of the immune system in the disease's pathogenesis.

25OHD has properties that promote differentiation, inhibit proliferation, and prevent apoptosis in brain cells. Offspring of mice with 25OHD deficiency have been found to exhibit increased cell proliferation in the brain (72). Cell proliferation is indeed associated with brain structural abnormalities, and 25OHD deficiency has been shown to reduce the expression of neurotrophic factors such as nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor in the brains of neonatal rats. This reduction could potentially lead to early brain dysfunction (73). Although MR results suggest a unidirectional effect of SCZ on 25OHD deficiency, this does not eliminate the possibility of a bidirectional relationship between these two factors. Given the intricate network of genetic correlations and pleiotropy, it is likely that the relationship between 25OHD and SCZ is multifaceted. One plausible explanation for this could be the complexity of SCZ etiology, which involves multiple genetic and environmental factors. SCZ pathogenesis is influenced by a wide range of environmental and lifestyle factors, including sunlight exposure, diet, and other socio-environmental elements, which play essential roles in vitamin D synthesis and metabolism (74–76). However, the potential positive effects of these factors on SCZ risk remain speculative (77). These factors add layers of complexity to SCZ etiology, making it unlikely that a single nutrient or metabolic factor could fully account for the disorder's development. In addition, vitamin D levels may play a more indirect or compensatory role rather than a primary causal role in SCZ. While our analysis found no direct causal link between 25OHD and SCZ, the inverse relationship where SCZ appears to influence 25OHD levels suggests that psychiatric conditions, lifestyle factors, or medication use in SCZ patients could negatively affect vitamin D metabolism or absorption. For instance, individuals with SCZ often have limited outdoor exposure and may exhibit poor dietary habits, both of which can lead to vitamin D deficiency (8, 78). Furthermore, antipsychotic medications, frequently prescribed for SCZ, have been shown to alter metabolic pathways, potentially affecting vitamin D levels (8). Several clinical randomized controlled trials have examined whether supplemental 25OHD levels improve symptoms of SCZ, but the results have been inconsistent. One study showed that vitamin D supplementation (50,000 IU vitamin D per week for 12 weeks) improved positive or negative symptoms of SCZ (79). Conversely, another study found no improvement in symptoms with vitamin D supplementation in patients using antipsychotic medications (80). Other small open-label studies have also shown inconsistent results (81, 82). The inconsistency of these trials may be attributed to varying baseline 25OHD levels, differences in patient profiles, or the impact of vitamin D on drug metabolism. Evidence suggests that vitamin D has a direct effect on drug metabolism by inducing CYP3A4, which may reduce the efficacy of antipsychotic medications that are CYP3A4 substrates (83). Studies have shown a negative correlation between serum vitamin D levels and the concentrations of such antipsychotics, indicating that while vitamin D may contribute to symptom relief (83), this effect could be counteracted by its impact on medication levels. Thus, while vitamin D supplementation remains important for overall health, particularly for those at risk of deficiency, current findings do not support its use as a primary or standalone treatment for SCZ. Instead, comprehensive approaches that consider the multifactorial nature of SCZ—including genetic, environmental, and lifestyle factors—are essential. For individuals at high risk of SCZ, strategies such as maintaining sufficient vitamin D levels through diet and sunlight exposure may offer general health benefits (9, 77), although their potential role in specifically reducing SCZ risk remains uncertain and requires further investigation. These lifestyle adjustments can help ensure adequate vitamin D, which may indirectly support health without the complexities introduced by direct supplementation in combination with antipsychotic treatment (84).

5 Limitation

This study has several limitations that should be considered. First, due to the use of summary-level data, we were unable to perform stratified analyses by key factors such as sex, age, or disease subtypes. While sex-specific effects were not explored, age-related differences and variations across SCZ subtypes could also influence the association between serum 25OHD levels and SCZ risk. Future studies utilizing individual-level data are necessary to assess potential interactions across these factors. Second, our analysis assumed a linear relationship between 25OHD and

SCZ, as current MR methodologies primarily focus on linear associations. Although non-linear MR methods are emerging, they are still in their early stages and have significant limitations, particularly when applied to summary-level data. As a result, we were unable to robustly assess non-linear effects, which may provide deeper insights into the 25OHD-SCZ relationship. Methodological advancements and access to individual-level data could help address these issues in the future. Furthermore, the study predominantly included individuals of European ancestry, which may limit the generalizability of the findings to other ethnic populations. Genetic variations and environmental factors, such as differences in vitamin D metabolism, may vary across ethnic groups. We have noted that while several associations in MAGMA gene-set analysis were initially observed, they did not retain statistical significance after adjustment for multiple comparisons. We caution readers to interpret these findings conservatively, recognizing that the non-significant adjusted *p*-values may indicate that these associations are not robust to stringent statistical correction. Additionally, we have highlighted the need for further validation in independent cohorts to confirm the findings. Future research should include more diverse populations to enhance the external validity of the results. Lastly, while the MR approach strengthens causal inference, it is not without limitations. Our analysis may be subject to horizontal pleiotropy, where genetic variants influence SCZ risk through pathways independent of 25OHD. Although sensitivity analyses were performed to address this, residual pleiotropy cannot be entirely ruled out. Additionally, MR relies on the assumption that the genetic instruments are valid proxies for the exposure, which may not always hold true in complex traits like SCZ. Ultimately, the robustness of our results requires further validation through experimental and mechanistic studies to fully confirm the causal pathways.

6 Conclusions

Our study has unveiled the genetic correlation between 25OHD levels and SCZ, further supporting the hypothesis that 25OHD deficiency and SCZ have shared genetic mechanisms. We have identified pleiotropic genetic loci and pathways that link these two conditions. Importantly, our Mendelian randomization analysis suggests that SCZ influences 25OHD levels. This research may serve as a foundation for future investigations into shared genetic mechanisms and molecular interactions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

G-WR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. X-ML: Investigation, Validation, Visualization, Writing – original draft, Formal analysis. H-ML: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. M-ZS: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. YJ: Investigation, Validation, Visualization, Writing – original draft, Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer Y-QY declared a shared parent affiliation with the author H-ML to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1415132/full#supplementary-material

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A narrative review focusing on randomized clinical trials of vitamin D supplementation for COVID-19 disease

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Current evidence is inconsistent on whether vitamin D supplementation can prevent COVID-19 infection or improve its clinical outcomes. To better understand and look into the issue, we went through the background knowledge of COVID-19 and vitamin D, searched in Pubmed [by using key words in the title containing "randomized clinical trial", "COVID-19", and "vitamin D (25-hydroxyvitamin D, or cholecalciferol, or calcidiol, or calcifediol) supplementation"] for publications of studies on vitamin D/supplementation in COVID-19 patients, especially those about the randomized clinical trials (RCTs). After reviewing these papers, we did a short background review of vitamin D and the pathophysiology of COVID-19, summarized the key features of the 25 RCTs in text and tabulated in a table of some of the features, commented, compared and discussed the differences between RCTs (for example, change the serum 25-hydroxyvitamin D concentration from nmol/L to ng/mL, making the comparison easier). The take-home question of the review is that serum 25-hydroxyvitamin D concentration is an important indicator of the supplementation effect of vitamin D correction but may not be reliable in predicting the supplementation effect on the clinical outcomes of COVID-19.

KEYWORDS

randomized clinical trial, vitamin D, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), COVID-19, serum 25-hydroxyvitamin D

Vitamin D metabolism and function

Vitamin D (VD) obtained by the body mainly comes in two structurally different forms: vitamin D2 (D2, ergocalciferol; from dieting) and vitamin D3 (D3, cholecalciferol; from exposing skin to sun and dieting), with D2 having a methyl group in C24 and a double bond in C22–C23 (Figure 1). Once in the body, they are converted by enzymes into 25-hydroxyvitamin D (25D; calcidiol, calcifediol), an inactive form of VD (most often measured and used as the indicator of serum VD level), and then hydroxylated to form the biologically active 1,25-hydroxyvitamin D (1,25D; calcitriol) (1). Once activated, VD binds to the nuclear VD receptor (VDR) and forms a heterodimeric complex with retinoic acid X receptor that recognizes specific DNA sequences (VD responsive elements), resulting in expression of VD responsive genes via a variety of transcription factors. Particularly, 1,25D is a factor involving in regulating and promoting calcium absorption and intracellular

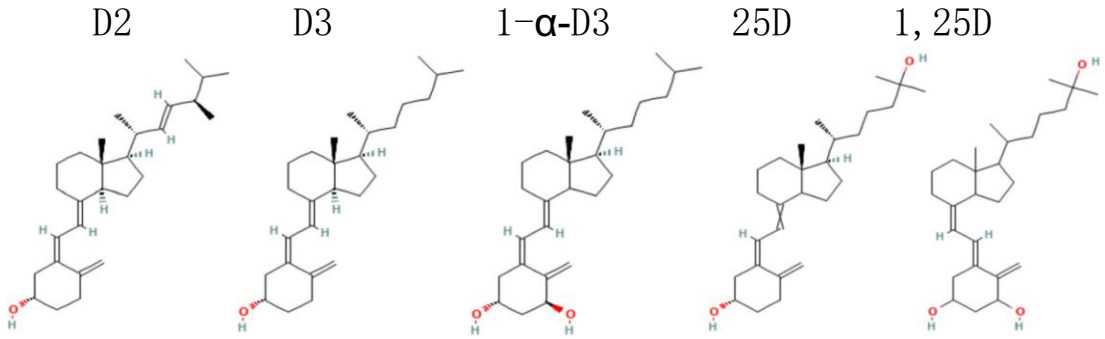

FIGURE 1
Maybe each of the 5 names above the 5 structures better in the midline of the corresponding structure. D2, vitamin D2, ergocalciferol; D3, vitamin D3, cholecalciferol; 1α-D3, 1-alpha-hydroxycholecalciferol, alfacalcidol; 25D, 25(OH)D, 25-hydroxycholecalciferol, calcidiol, calcifediol; 1,25D, 1,25-hydroxyvitamin D, 1,25(OH)2D, calcitriol.

transportation by increasing gene expression or concentration of the aforementioned proteins (2).

Approximately 3% of the human genome is under the control of 1,25D and regulated via the VD pathway (3). VDR is present in many human cells including various types of immune cells (such as dendritic cells, lymphocytes, macrophages, and monocytes), regulating the expression of a large number of target genes (~1,000) in these cells (4). Of the predicted 11,031 putative VDR target genes, more than 40% are assumed to involve in metabolism, about 20% in cell/tissue morphology, 10% each, respectively, in cell junction/adhesion, differentiation/development, and angiogenesis, and 5% with epithelial to mesenchymal transition (5).

Together, VD is a pleiotropic hormone and has profound impact on the human development, physiology, immunity through its connections to VDR.

COVID-19 pathophysiology

Although the first COVID-19 pandemic is over, its pathophysiology is still not fully understood (6). For severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to enter in the body and spread the infection, two sequential step reactions are required. The first step is SARS-CoV-2 invasion via host cell receptors (7), which requires S-protein priming to facilitate its entry of cells such as nasal, bronchial epithelial cells and pneumocytes (8). The second is cleavage of the spike protein by the transmembrane serine protease 2 (9).

Angiotensin-converting enzyme 2 (ACE2), one of the receptors for SARS-CoV-2, is most abundant in type II alveolar cells of the lungs, and also expressed in multiple tissue cells (airways, cornea, esophagus, ileum, colon, gallbladder, heart, kidney, liver, and testis) (10). ACE2 receptor-mediated SARS-CoV-2 infection initiates a cell signaling cascade, ultimately resulting in production of inflammatory cytokines, prothrombotic molecules, and acute phase reactants, which alone or together amplify the immune system's responses which will protect or damage the surrounding tissues.

Upon their initial entry, SARS-CoV-2 may subsequently migrate from the nasal epithelium to the upper respiratory tract via the ciliated cells in the conducting airways (8), and start to proliferate, and people infected at this stage are highly infectious with high viral load but may remain asymptomatic (11). Viral transmission at the pre-symptomatic stage significantly contributed to the pandemic (12).

If the hosts are able to cope and mount a strong interferon-mediated response at this early stage, they may control the viral replication and limit the disease severity (13). Although the precise mediators of early viral clearance are not yet completely understood, a critical role of interferons (IFN) in viral elimination is related to their potent antiviral activity and robust upregulation in mild COVID-19, considering type I IFNs (IFN-α, $-\beta$, $-\omega$) are indispensable in viral clearance (14). For patients failing to eradicate SARS-CoV-2 in its early stage, the disease may progress to the clinical phase or later stage of the infection, manifesting symptoms that may vary in severity and duration and resulting in a complex multisystem disorder (15).

Some proinflammatory cytokines are secreted following the binding and penetration of SARS-COV-2 into the respiratory epithelial cells (16). These cytokines include but not limited to: endothelial growth factor, granulocyte colony-stimulating factor (filgrastim), interferons (IFN-γ), chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, and CXCL10) and interleukins (IL1β, IL4, IL6, IL8, and IL10), macrophage inflammatory protein A, monocyte chemoattractant protein 1, tumor necrosis factor-α (17). Simultaneous increase of multiple inflammatory cytokines at a certain stage forms the 'cytokine storm', making it difficult to pinpoint the specific mediator of inflammatory response (18). Higher levels of different cytokine profiles have been determined among severe SARS-COV-2 patients (14).

Together, COVID-19 is a viral infection but has broad impact far beyond the respiratory/pulmonary system, and can potentially affect or even damage other systems through the vast distribution of ACE2 receptor and the circulating molecules.

VD deficiency/insufficiency is widespread

According to the Endocrine Society's Practice Guidelines (19) and "Vitamin D deficiency 2.0: an update on the current status worldwide" (20), 25D level at <20 ng/mL (50 nmol/L; according to https://unitslab.

com/node/84#google_vignette) is VD deficiency, 21–29 ng/mL (51–74 nmol/L) VD insufficiency, and ≥ 30 ng/mL (75–250 nmol/L) VD sufficiency. Of note that this criterion is for the consideration of maximum musculoskeletal health.

One study estimated that at a given time the VD concentration is suboptimal in half of the world's population across all age groups and residing in both developed and developing countries (21, 22). It is also estimated that globally there were more than one billion VD deficient people (23), illustrating that VD insufficiency/deficiency is a worldwide public health problem. For example, almost 25% of the subjects in USA had vitamin D deficiency (24), and 34.76% of a total of 227,758 participants in South America had vitamin D deficiency (25).

Interplay between VD and COVID-19

People, young and old, have witnessed and experienced the huge impact of the COVID-19 pandemic on our psychological/physical health and everyday life. Considering the wide distribution of VDR in and the profound impact of COVID-19 on the human body, the interplay between VD and COVID-19 might be far more complicated than what people have so far learned, and the exact pathways and mechanisms of their interplay are so far difficult to pinpoint. On the one hand, SARS-CoV-2 infection can cause an inflammation status leading to VD deficiency (26); on the other hand, VD deficiency might be a risk factor for COVID-19.

Soon after the emergence of COVID-19 at the end of 2019, a few researchers had suggested the connection of low VD with COVID-19, and some even hypothesized to use VD supplementation as an adjuvant therapy, for prophylaxis purpose to reduce COVID-19 severity, or even for a trial in COVID-19 patients (27, 28).

To better understand the background of VD-COVID-19 studies, about two dozens of work were also herein reviewed and the main results were summarized (Supplementary Table S1). Around ten studies show that VD insufficiency/deficiency was significantly related to the infection, severity, and mortality of COVID-19, in contrast to a few failing to show the connection. About equal number of studies either show or deny that VD supplementation helped in reducing ICU admission rate or mortality (Supplementary Table S1). A recent work supports the notion that VD deficiency (25D < 12 ng/mL) is an independent biomarker weathering the worsening of COVID-19, particularly in hospitalized non-severe patients (29).

Comparing to patients recovering without long COVID, those long COVID patients were found to have lower 25D levels (30). COVID-19 patients tend to have high prevalence of hypocalcemia (31), and daily D3 supplementation (either 2000 IU or 10,000 IU) for 2 weeks was able to increase the serum calcium level (32).

The first case study using VD supplementation in COVID-19 patients was conceived soon after the outbreak, started in April 2020, and finished half year later. Four VD insufficient/deficient patients diagnosed with COVID-19 were given with either daily D3 (1,000 IU, standard dose) or D2 (50,000 IU, high dose) for 5 days (33). The 25D baselines of all four patients were < 22 ng/mL, bordering between insufficient/deficient level. On day 6, the serum 25D of the two patients receiving high dose D2 reached 39.9 ng/mL and 50.5 ng/mL respectively, contrasting to the minimally changed level of the other two receiving standard dose D3.

The results of the first of the 25 reviewed RCTs were published in August 2020 (discussed below), and the past 4 years brought in more results from more studies of different natures (larger scale, randomized, single or double blinded, with control/placebo group), with RCTs being the main focus of this review.

Key features of the 25 randomized clinical trials (RCTs #1–25) of vitamin D supplementation for COVID-19 disease

To make the manuscript concise, the following abbreviations for various types of vitamin D will be used: VD, vitamin D; D3, vitamin D3, cholecalciferol; ERC, extended-release calcifediol; 1α-D3, 1-alpha-hydroxycholecalciferol, alfacalcidol; 25D, 25(OH)D, 25-hydroxycholecalciferol, calcidiol, calcifediol; 1,25D, 1,25-hydroxyvitamin D, 1,25$(OH)_2$D, calcitriol.

For similar reasons, the following abbreviations are used below: ARDS, acute respiratory distress syndrome; BMI, body mass index; CRP, C-reactive protein; CT, computed tomography; ICU, intensive care unit; CLIA, chemiluminescent immunoassay; d, day(s); ELIA, electrochemiluminescence immunoassay; h, hour; HPLC, high-performance liquid chromatography; LC-(T)MS, liquid chromatography (tandem) mass spectrometry; PaO2/FIO2, partial pressure to fractional inspired oxygen; rRT-PCR, real-time reverse transcription polymerase chain reaction; rSOFA, respiratory Sepsis related Organ Failure Assessment; WHO, World Health Organization; y, year(s).

#1 (34): 76 Spanish hospitalized patients (mean age: 53 y) were enrolled in the 'Pilot Covidiol' study (NCT04366908). Their COVID-19 infection diagnoses were made by radiographic patterns of viral pneumonia and by positive SARS-CoV-2 PCR. The sample size was calculated based on certain assumptions. The 25D serum levels at enrollment or after VD supplementation were not reported. The control group had a higher percentage of hypertension at enrollment.

All participants had 'standard' of COVID-19 treatment (the best therapy available at that time per hospital protocol, a combination of hydroxychloroquine and azithromycin). The VD group (50 patients) received the first oral dose (0.532 mg) of calcifediol on the day of admission, followed with 0.266 mg on d 3 and d 7, and then weekly until discharge or ICU admission (calcifediol is 25D; according to https://vitamored.com/products/vitamored-vegan-vitamind3-calcifediol, 0.532 mg of calcifediol = 106,400 IU of D3). The rate of ICU admission and deaths were the prespecified outcomes of effectiveness of treatment. When compared to those without 25D addition (50%, 13 in total), the need for ICU admission of the 25D-supplemented patients (2%) was significantly reduced.

#2 (35): A small group (n = 40) of asymptomatic or only mildly symptomatic COVID-19 patients without comorbidities were enrolled in the SHADE study in India (NCT04459247), 14 of them were randomized to receive placebo (5 mL distilled water), and 16 to receive daily 60,000 IU of cholecalciferol (D3; oral nano-liquid droplets) from the first day of enrollment until their serum 25D reached the goal (>50 ng/mL) by d 14. Although only 40, the sample size was well calculated. For those reaching the goal earlier by d 7, they continued to receive 60,000 IU at d 14. The proportions of participants

who turn SARS-CoV-2 negative (confirmed twice at 24 h interval) before week 3 was set as the primary outcome, and the other outcome was the change in the level of inflammatory markers after treatment. At d 0 and 7, 25D levels were assessed by ELIAusing a supplied kit. Oro-pharyngeal swabs were obtained at six time points (days 5, 7, 10, 14, 18, 21) and SARS-CoV-2 RNA detection was performed by RT-PCR.

The serum 25D level in ten out of the 16 patients achieved >50 ng/mL by d 7 and another two by d 14; 2 weeks of D3 supplementation raised the median serum 25D from 8.6 ng/mL to 51.7 ng/mL ($p < 0.001$). Greater proportion of the VD supplemented patients turned their viral RNA tests negative (62.5% vs. 20.8% in control group, $p < 0.018$), and with a significant decrease in fibrinogen ($p = 0.007$).

#3 (36): 69 mild to moderate COVID-19 hospitalized patients (20–75 y) who were newly diagnosed (no more than 3 d by RT-PCT) were enrolled in a trial from 29 July–22 September 2020 in Saudi Arabia to orally take either a high (5,000 IU, $n = 36$) or low (1,000 IU, $n = 33$) daily D3 supplementation for 2 weeks. A mild–moderate COVID-19 case was defined by the Saudi Ministry of Health that the patient on presentation had clinical symptoms and required supportive care but not oxygen. Serum 25D was assessed using the CDC-approved CLIA assay as certified by the VD Standardization-Certification Program (VDSCP); Of note, the VD baseline of 40 cases (55%) was within the deficiency level.

Only the 5,000 IU treatment significantly increased 25D levels (baseline 53.4 nmol/L = 21.36 ng/mL vs. after supplementation 62.5 nmol/L = 25 ng/mL; $p = 0.001$ without adjustment, and $p = 0.003$ after adjustment for covariates: age, sex, baseline BMI, and D-dimer). Only the high dose (but not 1,000 IU) D3 supplementation shortened the time to recovery from cough and gustatory sensory loss, with caveats that the 1,000 IU group (25D level: baseline 63 nmol/L = 25.2 ng/mL vs. after supplementation 59.9 nmol/L = 23.96 ng/mL) had significantly higher BMI ($p = 0.02$) at enrollment and significantly older ($p = 0.03$). A significant increase in neutrophil ($p = 0.03$) and urea ($p < 0.001$) was noticed in the high dose group after supplementation.

#4 (37): 321 PCR-negative Mexican healthcare workers highly exposed to COVID-19 prior to vaccination were enrolled between July 15 to December 30 of 2020, and half of them were randomly assigned either to receive D3 or placebo (capsules with identical appearance containing 450 mg cornstarch). Sample size (156 subjects per group) was calculated based on a binary result, and randomization was done by using a software (Research Randomizer; https://www.randomizer.org/). The placebo group was older and had a higher frequency of diabetes than the supplementation group. Serum 25D and antibody tests were measured at baseline and at d 45, using a Waters ACQUITYH UPLC Class coupled to a Xevo TQD, with an APCI Ion SABRE II probe and a solid phase extraction cartridge. Chromatographic analyses were performed with a C18-column at 50°C. The prespecified primary outcomes were the rate of SARS-CoV-2 infection by RT-PCR tests and the severity of the disease. Secondary endpoints were set as the reduction of VD deficiency prevalence and the frequency of treatment-associated adverse events.

Regardless of the baseline level (mean 18.3 ng/mL), D3 supplementation (4,000 IU daily for 30 d) significantly increased the serum 25D concentration at d 45 and lowered the COVID-19 infection rate (6.4% D3 group vs. 24.5% placebo group, $p < 0.001$) in the follow-up period (days 7, 14, 21, 28 and 45), after adjusting for a few factors (age, comorbidities, vitamin D deficiency at baseline, as well as by site of study and type of personnel). By multivariate analysis, the authors could predict COVID-19 risk through Delta serum 25D concentration.

#5 (38): A multicenter trial (NCT04344041) enrolled 254 French COVID-19 patients (median age: 88 y) between April 15 and December 17 of 2020, and within 72 h after the diagnosis gave some of them (the intervention group) orally a single D3 treatment on the day of inclusion administered under medical supervision (ideally during food intakes for better absorption) to compare the 14 d overall survival between the high (400,000 IU) and standard dose (50,000 IU) groups. Patients positive in RT-PCR test and/or chest CT scan were allocated by dynamic randomization using a minimization algorithm and considering 6 criteria. All patients had at least one of COVID-19 worsening risk factors (age ≥ 75 y, SpO2 $\leq 94\%$, or $PaO_2/FiO_2 \leq 300$ mm Hg). 14 d overall mortality was the prespecified primary outcome, calculated after adjusting for randomization strata (age, delirium, hospitalization, ongoing cancers, oxygen requirement, profuse diarrhea, sex, and use of medications). Secondary outcomes were between-group comparison of safety, overall mortality within 28 d after enrollment, and mortality at both time points (14 and 28 d). Three follow-up visits (at 7, 14, and 28 d) were scheduled after the randomization. Blood samples from baseline (before D3 administration) and d 7 (±1 d) were obtained in the morning. Serums from thawed samples (within 4 h) were analyzed locally at each site to measure changes in serum 25D concentration by CLIA. Immunoassay kits recognize both D2 and D3. No participants were in the ICU at the time of entering the trial.

The difference of the serum 25D concentration at baseline was not significant between groups (high dose 53.0 nmol/L = 25.2 ng/mL vs. standard dose 43.0 nmol/L = 21.36 ng/mL), but the serum 25D level of the high dose group after week long supplementation was significantly higher ($p < 0.001$) than the standard dose group (150.5 nmol/L = 60.2 ng/mL vs. 64.5 nmol/L = 25.8 ng/mL). Compared to the standard dose, the high dose treatment reduced the overall mortality at d 14 (unadjusted $p = 0.20$; adjusted $p = 0.049$) but not after 28 d. The weakness of absence of placebo group was partly compensated by controlling for imbalances of randomization strata and prognostic factor baselines. Even though the 25D level (150.5 nmol/L = 60.2 ng/mL) after 400,000 IU D3 intervention was relatively high, the protocol-specified adverse events of interest (37 items) were not significantly different between the two groups. Notably, corticosteroids were prescribed for 34 participants (27%) in the high-dose group and 41 (32%) in the standard-dose group.

#6 (39): Belgian (Caucasian, VD deficiency defined as serum 25D concentration ≤ 20 ng/mL and hospitalized for confirmed SARS-CoV-2 infection at screening) COVID-19 patients of unspecified severity were enrolled in the trial (NCT04636086) from August 2020 to August 2021. Participant sample size was not formally calculated. The trial lasted for a maximum of 9 weeks (up to 6-week treatment period and a maximum of 3-week follow-up period). The severity of the disease was assessed by the ordinal WHO scale for clinical improvement both at randomization and efficacy evaluation. The study treatment was under the supervision of the clinical staff, leading to 100% compliance. The last day of the 6-week treatment period was the last day of hospitalization or d 36, whichever was first. Prespecified outcomes of effectiveness included 25D serum level, ordinal scale for clinical improvement as recommended by the WHO, hospitalization

length, intensive care unit admission, time until absence of fever, need for supplemental oxygen, non-invasive ventilation, high-flow oxygen devices, invasive mechanical ventilation or additional organ support and death. The Fujirebio 25-OH VD assay on Lumipulse G1200 analyzer was used to screen the 25D concentrations, which showed excellent concordance with the LC–MS/MS method used in the laboratory and provided results in a fast turnaround time, fitting the needs of a screening. To rapidly restore the D3 level, 4 consecutive daily VD doses of 25,000 IU were first given to patients of the intervention group, and 25,000 IU per week (up to 6 weeks) to maintain the 25D level. To assure a standard VD supplementation to those with possibly more severe VD deficiency, all ICU patients with enteral nutrition would additionally receive 600 IU VD per day.

D3 supplementation increased their serum 25D level from below 20 ng/mL to 29.9 ng/mL at the end of the study, improved the clinical outcome (clinical recovery, hospitalization length, supplemental oxygen duration). The median length of hospital stay significantly decreased in the VD group compared to the placebo group (4 d for the VD group vs. 8 d for the placebo group; $p = 0.003$); and none of the patients treated with VD were hospitalized after 21 d compared to 14% of the patients treated with placebo. Among all the patients who needed supplemental conventional oxygen, the administration of VD significantly decreased the duration of treatment (4 d vs. 7 d; $p = 0.012$). At d 7, 71% of the patients supplemented with VD switched from the moderate to the mild category of the scale compared to 18% in the placebo group ($p = 0.0048$). At d 36, 90% of the patients from the VD group were no more infected compared to 77% in the placebo group. There was no effect of age, arterial hypertension, BMI, cardiac pathology, diabetes, gender, height, hepatic failure, vaccinal status and weight on the primary endpoint ($p > 0.05$).

#7 (40): 45 moderate COVID-19 Mexican children (81% had some comorbidity, nearly half were obese) who required hospitalization and supplemental oxygen were enrolled in the trial (NCT04502667) and their disease severity was accordingly classified as mild, moderate, severe or critical. Patients were randomly assigned by a researcher to the VD supplementation group or to the control group not receiving VD. Supplementation started on the day of enrollment by receiving a daily 1,000 IU D3 for children <1 y or 2000 IU for those 1 to 17 y old, and continued during hospitalization for a minimum of 7 d and a maximum of 14 d. The prespecified outcome variables were progression of oxygen requirement, development of complications, and death.

The trial measured the baseline serum 25D levels (median, 13.8 ng/mL in the VD group and 11.4 ng/mL in the control group) using the Fujirebio 25-OH VD assay on Lumipulse G1200 analyzer but without the endline data. The trial was designed to last from 24 March 2020 to 31 March 2021 but stopped prematurely after seeing that none of the basal VD values of the patients were at normal levels, and for ethical reasons decided to supplement VD to all hospitalized COVID-19 patients. The original outcomes were set to measure the progression of oxygen requirement, the development of complications, and death.

#8 (41): After power calculation for sample size, 116 Egyptian patients (mean age ≈66 y) hospitalized with pneumonia (verified by chest CT scan, a positive COVID-19 RT-PCR and a hyperinflammation status) were enrolled in the trial (NCT04738760; From Dec. 2020 to June 2021) and allocated using a table of random numbers to receive either low (oral 1 mcg of 25D/d for five d; 1 mcg 25D equals to 200 IU D3 as per https://vitamored.com/products/vitamored-vegan-vitamind3-calcifediol) or a single high dose D3 treatment (200,000 IU intramuscularly). Serum 25D levels were not reported. D614G mutant strain was detected in patient samples, which was prevalent in late 2020. The following treatment was also given to all patients per day, for at least five d: 25 mg quetiapine (bedtime), 4 g paracetamol (1 g every 6 h), 6 mg dexamethasone, 400 mg hydroxychloroquine, 400/100 mg lopinavir/ritonavir twice, or 200 mg remdesivir loading dose followed by 100 mg.

The prespecified primary outcome was set as improvement of oxygenation parameters. Numerous secondary outcomes were set, including: hospital stay length, mortality, variation in inflammatory markers (CRP, ferritin, and lactate dehydrogenase), and occurrence of secondary infections and adverse event.

The single D3 high dose was found to be associated with better clinical improvement (clinical improvement, length of hospital stay, need for high oxygen, need for a mechanical ventilator or non-invasive mechanical ventilator, the occurrence of sepsis) and fewer adverse outcomes compared to low-dose VD group. Compared to the low-dose group, fewer patients in the high-dose VD group needed an invasive mechanical ventilator ($p = 0.03$), required ICU admission ($p = 0.016$), showed secondary bacterial infection in the form of sepsis ($p = 0.04$), had a decrease in basal CRP value ($p = 0.007$), and more patients showed clinical improvement ($p = 0.03$).

#9 (42): 134 mild to moderate COVID-19 patients in USA were enrolled in the REsCue trial (NCT04551911) to take ERC (extended-release calcifediol, with a lipophilic fill gradually releasing 25D) by an initial big dose (300 mcg on d 1–3; 300 mcg equals to 60,000 IU as per https://vitamored.com/products/vitamored-vegan-vitamind3-calcifediol) and follow by a maintenance dose (60 mcg on d 4–27; 60 mcg equals to 12,000 IU of D3). Participants were recommended to remain fasting for 3 h after dosing. Serum total 25D was analyzed by LC–MS, and total 1,25D by CLIA.

Thirty-four symptoms were self-reported daily using the FLU-PRO Plus questionnaire (an outcome tool validated for respiratory tract viral infections), positive for SARS-CoV-2 within the previous 3 d via RT-PCR or substitutable FDA-authorized test; mild to moderate COVID-19, defined as the absence of clinical signs indicative of more severe disease such as oxygen saturation < 94% or respiration rate > 30 breaths per minute. The two specified primary end points were attainment of the targeted serum 25D level by d 14, and time to resolution of five composite COVID-19 symptoms (trouble breathing, chest congestion, body aches or pains, chills or shivering, dry or hacking cough) which were part of the chest/respiratory and body/systemic domains of the questionnaire for which mean scores of ≥1.5 were required for enrollment. Secondary end points included time to resolution of each composite symptom and of aggregated symptoms as a function of serum 25D.

This dosing strategy raised serum 25D from 37 ng/mL to 82 ng/mL ($p < 0.0001$) by d 7 and remained elevated to the end of the study (d 28), and accelerated the resolution of respiratory symptoms and mitigated the risk for pneumonia. In the full analysis set (FAS), 81% of patients in the ERC group achieved 25D levels of ≥50 ng/mL versus 15% in the placebo group ($p < 0.0001$), respiratory symptoms resolved 4 d faster when 25D was elevated above baseline level at both d 7 and 14.

#10 (43): Supplementation with alfacalcidol (1α-25D, a synthetic analogue of 25D) in addition to standard care for COVID-19 was

applied to Thai COVID-19 pneumonia patients (≥ 18 years) in the trial (TCTR20210906005) between July 2020 and March 2022, with the supplementation starting on the day of enrollment until the end of hospitalization. Of note, some patients were diagnosed with pneumonia either on admission or developed pneumonia later. Among the 241 patients, 43.57% were VD insufficiency (105 patients), 26.56% VD deficiency (64 patients), and 5.39% severe VD deficiency (13 patients). All patients received antiviral therapy, and over half of the participants used corticosteroids [i.e., 76/147 patients (51.70%) in the control group and 82/147 patients (55.78%) in the intervention group].

The prespecified clinical outcomes were set as: pneumonia treatment duration, length of hospital stay, and change in pneumonia severity index between enrollment and discharge. The secondary outcomes were subgroup analyses according to the need for supplemental oxygen, 25D concentration (< 12 and < 20 ng/mL), prednisolone administration (≥ 1 mg/kg/d), lymphopenia (absolute lymphocyte count, < 1,000 cells/mm^3), and CRP concentration (< 30, ≥ 30, ≥ 40, and ≥ 50 mg/L).

It is odd the authors reported the baseline 25D level at enrollment (supplementation group 22.50 ng/mL vs. placebo group 20.83 ng/mL, both within the insufficiency range 20–29.99 ng/mL) but not the level after supplementation. 1α-25D supplementation was not beneficial to all patients, only benefited those who required supplemental oxygen or received high-dose corticosteroid therapy or had high CRP concentrations (> 30 mg/L) at the time of treatment initiation.

#11 (44): In this international, multicenter trial (ACTRN12620000557932) carried out between Jan. and June 2021, D3 (5,000 IU daily for 14 d) was given as a combo (together with hydroxychloroquine, azithromycin, zinc, with/without vitamin C) to COVID-19 hospitalized patients. COVID-19 patients were first diagnosed at the time of enrolment by PCR testing via nasal and/or oral swab. 73% of the patients had comorbidities, ranging from diabetes (35%), heart disease (36%) to lung disease (34%). None of the patients had optimal VD levels (≥75 nmol/L = ≥30 ng/L); specifically, 55% of them were severely deficient (<25 nmol/L = <10 ng/L), 42% deficient (<50 nmol/L = <20 ng/L), and only 3% at insufficient level (<75 nmol/L = <30 ng/L). Serum 25D levels after supplementation were not reported. D3 was not controlled (i.e., no D3-alone group), therefore the role of D3 could not be certain. The primary outcome was mortality or need for invasive mechanical ventilation within the first 15 d from enrolment, and the secondary outcome includes the WHO Master Protocol ordinal score at d 15.

Nevertheless, the study concluded that the combo protocol was safe and effective in treating COVID-19 infection, and VD deficiency to be a high-risk factor of severe COVID-19 disease and hospitalization. The lower the vitamin D level, the higher the probability of being admitted to the ICU (14.2 nmol/L = 5.7 ng/mL vs. 25.1 nmol/L = 10 ng/mL, $p < 0.0001$). Furthermore, a statistically significant correlation was found between lower baseline VD levels and longer hospital stay ($p = 0.003$).

#12 (45): 50 mild to moderate (not yet so severely-ill to require hospital admission) and RT-PCR confirmed COVID-19 patients were enrolled in Pakistan between 2 Sep. 2021 and 28 Nov. 2021, and randomization was carried out using computer-generated random number tables. Among other outcomes for this pilot study, the primary outcome was set as patient testing negative for SARS-CoV-2 in the RT-PCR analysis, and the secondary outcomes included improvement in the COVID-19-associated acute symptoms, and laboratory biochemistry.

Compared to the control group using standard of care only (including paracetamol with or without azithromycin), the combo (co-supplementation of 360 IU D3 together with curcumin and quercetin) cleared the SARS-CoV-2 viral infection faster and relieved the acute symptoms quicker at the end of supplementation for 14 d, probably by modulation of the early-stage hyperinflammatory response. Serum 25D levels were not reported, and no definite role of D3 could be certain since no D3 alone group was designed in the trial (NCT05130671).

#13 (46): 120 mild to moderate COVID-19 patients tested positive in SARS2-CoV-2 PCR were enrolled in the trial (NCT04981743) after the sample size was calculated (GPower v.3.1.9.4). D3 (2,000 IU) was given to the D3-alone group and to the *Nigella sativa*-D3 combination group in addition to the standard therapy to see the supplementation effect on the clinical outcome. Serum 25D levels were not measured. The main outcomes of this study were the viral clearance judged by a negative PCR test result and the symptom alleviation during the duration of 14 d. Patients tested negative on d 7 were considered having cleared of the virus. Negative COVID-19 results by PCR test were recorded on the 7th and 14th d of therapy.

The *Nigella sativa*-D3 combination group was superior compared to those of the other studied arms, and the independent contribution of D3 supplementation could not be certain. Comparing to the control group, the other groups had reduced severity of cough, diarrhea, fatigue, and pharyngitis. However, the four groups showed non-significant relief of symptoms (ageusia, anosmia, headache, rhinorrhea, shortness of breath, and vomiting). VD3 group showed an increase in lymphocyte count (137/μL) and total leukocyte count (1.17×10^3/μL) at the end of the study period.

#14 (47): 181 COVID-19 Indian patients were enrolled in the trial (NCT04641195) from April 2021 and ended in Feb. 2022 after the sample size was calculated using methodology for survival times, and randomization was done by an independent statistician. Infection was confirmed by rapid antigen test or RT-PCR.

The prespecified primary outcome was set as the time to resolve cough, fever, and shortness of breath. The secondary outcomes included: duration of individual symptoms and hospital stay; need for assisted ventilation; all-cause mortality; and blood biomarkers (nutritional, inflammatory, and immunological markers). Hospital staff oversaw inpatient participants taking their daily supplements or reminded those who left the hospital to take their supplements during regular telephone follow-ups (completed in August 2022). Participants were followed either daily in person if in hospital or every 3 d via telephone (upon leaving the hospital) for 8 week to collect data on COVID-19 symptoms, supplement compliance, and any adverse events.

D3 supplementation (180,000 IU bolus at enrollment, then 2000 IU daily from the 2nd d for 8 weeks) did not improve COVID-19 treatment outcomes (resolution of cough, fever, and shortness of breath).

#15 (48): The CORONAVIT program (NCT04579640) lasted more than one y (from 1 May 2020 to 6 Oct. 2021), enrolled 6,200 cohort participants (about 95% were white people, and about 90% resided in England), the largest scale among the 25 RCTs, after the sample size was calculated on certain assumptions and by using a graphical user interphase, and randomization was done by using a

computer program (Stata v14.2). For 6 month, it delivered daily either low (800 IU, $n = 1,550$) or high (3,200 IU, $n = 1,550$) of D3 to UK residents (≥16 y) whose blood 25D concentrations were under 75 nmol/L (30 ng/mL).

The primary outcome was the proportion of participants with acute respiratory tract infection of any cause confirmed by a doctor or a swab test. Secondary outcome was the proportion of COVID-19 participants confirmed by a swab test.

25D concentrations were measured using a blood spot testing kit testing the capillary blood, and concentrations of 25D3 and 25D2 were determined in dried blood spot eluates using LC-TMS after derivatisation and liquid–liquid extraction [good overall agreement was observed between using the blood spot method and plasma 25D concentrations in paired capillary and venous samples, showing a minimal overall bias of −0.2% (bias range − 16.9–26.7%)].

2,674 (86.3%) of the intevention group had baseline 25D concentrations <75 nmol/L (<30 ng/mL, defined as deficient or suboptimal). Compared to baseline level (66.6 nmol/L = 26.64 ng/mL), both supplementation strategies significantly increased the mean 25D concentrations (low dose group 79.4 nmol/L = 31.76 ng/mL, high dose group 102.9 nmol/L = 41.16 ng/mL) measured after 6 month VD supplementation but none reduced the risk of COVID-19. The incidence or severity of acute COVID-19 or prolonged symptoms were not statistically and significantly different between the low or the high dose group compared with the no supplementation group.

#16 (48): 120 Brazilian patients (over half were white, 30% mixed ethnicity, 10% black) with moderate to severe COVID-19 infections were enrolled from June 2 to August 27 of 2020 in the trial (NCT04449718) to receive a bolus single dose of D3 (200,000 IU). The mean time from the onset of symptoms to randomization was 10.3 d, and from hospitalization to randomization was 1.4 d. At the time of enrollment and at some point during the hospital stay, patients had their COVID-19 diagnoses confirmed by PCR testing or ELISA to detect IgG against SARS-CoV-2. Overall, 125 of 210 patients (59.5%) had CT scan findings suggestive of COVID-19 and 147 of 237 (62.0%) had a PCR test result positive for SARS-CoV-2.

Quantitative outcomes were assessed at baseline when enrolled and compared to those when discharged. 212 (89.5%) required supplemental oxygen at baseline. The primary outcome was set as the length of hospital stay; and the secondary outcomes were prespecified as: creatinine, CRP, serum levels of 25D, total calcium; mortality during hospitalization; the number of patients admitted to ICU or required mechanical ventilation and the duration of mechanical ventilation. 25D were assessed by CLIA.

The week long supplementation significantly increased the mean serum levels of 25D from 20.9 ng/mL to 44.4 ng/mL but did not significantly reduce hospitalization length. Among the patients with 25D deficiency at baseline, no significant differences were observed in the median hospital length of stay between the D3 and placebo group, which was in sharp contrast to #17.

#17 (49): Between April 4 of 2020 to April 22 of 2021, the multicentre international COVID-VIT-D program (NCT04552951) enrolled 548 moderate–severe COVID-19 patients from four countries (Argentina, Chile, Guatemala and Spain) and gave the treatment group an oral bolus of 100,000 IU of D3 at hospital admission beside standard care. Criteria for hospitalization included lung radiological evidence of characteristic COVID-19 disease (e.g., bilateral multifocal ground-glass opacities >50%), and/or moderate–severe flu-like symptoms (e.g., having oxygen saturation lower than 94%), and/or comorbidity. 83.1% of the admitted patients had pulmonary involvement. The most frequent symptoms were fever (71.5%), cough (66.5%), weakness (62.2%), dyspnoea (54.0%) and headache (34.6%); and the most frequent comorbidities were hypertension (43.8%), diabetes (24.7%) and cardiovascular disease (21.2%). Serum D3 were measured at the time of hospital admission locally in each center by ECLIA or CLIA, and differences of baseline serum D3 by countries were observed (median ng/mL; Argentina 16.0; Chile 19.5; Guatemala 24.1; Spain 13.4).

Three outcomes were set as the end points of the COVID-VIT-D trial: length of hospitalization, admission to the ICU and mortality. Although the supplementation raised their serum 25D from 17.0 to 29.0 ng/mL (at discharge), slightly shy from the optimal level (>30 ng/mL), it did not improve the COVID-19 outcomes. Interestingly, those with relatively high baseline serum D3 level (>25 ng/mL) were associated with a lower risk of pulmonary involvement and ICU admission, and less days of hospitalization, comparing to those with low level (≤10 ng/mL).

#18 (50): Aiming to evaluate whether VD supplementation could prevent respiratory worsening among hospitalized patients with COVID-19, this multicentre CARED trial (NCT04411446) enrolled 218 mild-to-moderate COVID-19 patients in Argentina between August 2020 and June 2021, and gave the intervention group a single high oral dose (500,000 IU) of D3 as soon as possible after randomization.

There were no significant differences between treatment groups in baseline characteristics. The primary outcome was set as change in the respiratory SOFA score between baseline and the highest value recorded up to day 7; and three secondary outcomes were ICU admission, the length of hospital stay, and in-hospital mortality. Values of ratios SpO2/FiO2 were used to calculate the SOFA scores. Risk factors included: hypertension (43.1%, $n = 94$), obesity (39.9%, $n = 87$), diabetes (26.6%, $n = 58$), chronic respiratory disease (11.9%, $n = 26$), and cardiovascular disease (4.6%, $n = 10$).

The serum 25D concentrations increased from 32.5 ng/mL at baseline to 102.0 ng/mL (7 d after supplementation) but did not prevent the respiratory worsening and had no significant effects on the length of hospital stay or other outcomes.

In the first stage, the study aimed to assess the effects of VD on SOFA, and the second stage aimed to evaluate the effects of VD on clinical events. Enrolled patients were admitted to general wards within the last 24 h, with SARS-CoV-2 confirmed infection by RT-PCR, an expected hospitalization for at least 24 h, oxygen saturation ≥ 90% (measured by pulse oximetry breathing ambient air), and at least one of the following conditions [age 45 or older or asthma (at least moderate), body mass index ≥30, chronic obstructive pulmonary disease or, cardiovascular disease (history of myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting or valve replacement surgery), diabetes, or hypertension]. During the first 7 d blood pressure, heart and respiratory rate, inspired fraction of oxygen, SpO2, temperature, and clinical and adverse events were recorded. Serum 25D levels were determined quantitatively by CLIA in a central laboratory.

The Steering Committee decided to stop the recruitment and terminate the trial on 7th July 2021 based on that the differences between groups, either on the primary outcome (i.e., the change in SOFA) and the secondary outcomes, did not meet the prespecified criteria to proceed to the second stage.

#19 (51): The study (NCT05166005) lasted from 30 Nov. 2020 to 20 March 2021. Two doses of bolus D3 supplementation (50,000 IU on the 1st and the 8th d of hospitalization) were given to hospitalized COVID-19 patients in Russia. The COVID-19 diagnosis was confirmed by PCR-test and/or chest CT scan, and the disease severity (mild, moderate, severe) was judged accordingly. Serum 25D levels were measured using a CLIA on microparticles. Of note, the control patients were significantly younger than VD group patients ($p = 0.03$), otherwise they were comparable and had no significant differences in baseline parameters.

The primary outcomes were set as changes of the following parameters between the first d and 9th d of hospitalization: serum 25D and CRP levels, complete blood count and B cell subsets. The secondary endpoints were set to evaluate the effects of D3 supplementation on ICU admission rates, and clinical outcomes (disease severity, hospitalization duration and oxygen supplementation).

The supplementation increased the serum 25D level by 40.7% [still shy from the recommended 25D level (40 to 60 ng/mL)], and the level on the 9th d was found negatively associated with the number of bed d ($r = -0.23$, $p = 0.006$), but no other differences (ICU admission rates, mortality, and the average time of hospital stay) were found between the supplemented and control groups. Immunologically, the supplementation group had significantly higher counts of neutrophil ($p = 0.04$), lymphocyte ($p = 0.02$), and $CD27^{-}CD38^{-}$ double negative B cells ($p = 0.02$), but lower CRP ($p = 0.02$ at the 9th d of hospitalization) and frequencies of $CD38^{++}CD27$ transitional and $CD27^{-}CD38^{+}$ mature naive B cells ($p = 0.006$ and $p = 0.02$). Thus, VD supplementation raised 25D level and affected immunity, which might contribute to change their course of COVID-19 in VD insufficient patients.

#20 (52): After sample size was calculated using Package "Medcalc" (trial version) and random allocation sequence generated using Microsoft Excel, 117 Tunisian patients (mean, 42 y; 65.8% of the participants were asymptomatic) who remained RT-PCR positive for COVID-19 on the 14th d were enrolled in the trial (NCT04883203), and 57 of them received a single dose of 200,000 IU of D3. The intervention was made by medical residents, starting from May to August 2020. The primary outcome was set as the recovery delay (defined as the period between the day of the 14th RT-PCR-positive result and the day of the second successive negative RT-PCR test result), and secondary outcomes were set to monitor the changes of SARS-CoV-2 RT-PCR cycle threshold values between that at the beginning (date of randomization) and the second successive negative RT-PCR test.

Compared to the placebo group, this bolus D3 supplementation had not shortened the recovery delay. Conversely, the median duration of RNA viral conversion was significantly longer in the D3 supplementation group than in the placebo group. One of the limitations of this study, as the authors admitted, is that 25D serum levels was not measured, neither at enrollment nor after VD supplementation.

#21 (53): COVID-19 PCR positive patients needing invasive or non-invasive respiratory support were eligible for inclusion in the trial (NCT05384574), and 155 severe (on respiratory support) COVID-19 patients admitted to ICU in a Croatian hospital were enrolled and randomized using a computer-generated code to receive D3 supplementation (10,000 IU daily for 14 d). All patients included in this study received standard care. Mechanical ventilation was applied with protective lung ventilation using tidal volumes 4–8 mL/kg and plato pressures ≤30 cm H_2O.

Dexamethasone was routinely administered to all eligible patients. Number of days spent on respiratory support (invasive or non-invasive) was set as the primary outcome; secondary outcomes included: all-cause mortality on d 14, 28 and 60, clinical improvement at d 28 (according to WHO clinical progression scale), number of days spent in ICU, number of days spent in hospital, bacterial superinfections, neutrophil to lymphocyte ratio and disease severity (CRP levels, PaO_2/FiO_2 ratio, D-dimer levels, fibrinogen, ferritin, PCT).

All these patients had vitamin D levels measured on admission. VD supplementation began within 48 h of admission to ICU and last for at least 14 d during ICU stay or anywhere else orally or via gastric tube by experienced nursing staff. Disease severity markers were collected daily during ICU stay and every third d after discharge from ICU. VD levels were checked three times (on admission to the ICU, d 7 and 14; of note, VD levels were not measured for patients in the control group on d 7 and 14), and measured in a hospital laboratory using the ECLIA method.

The mean 25D level before supplementation (on admission) was 27.1 nmol/L (10.8 ng/mL), which reached 38.5 nmol/L (15.4 ng/mL) and 56.2 nmol/L (22.5 ng/mL), respectively, on d 7 and 14 after supplementation. The supplementation had not made a difference in either the main (days on respiratory support) or any of the secondary outcomes (days spent in ICU or length of hospital stay). The sample size was shy from the calculated one to detect a 2-day difference in number of days on respiratory support (137 patients in each group) due to short of patients admitted.

#22 (54): A total 106 hospitalized Iranian patients who had a circulating 25D concentration of <30 ng/mL and in need of respiratory support were enrolled in this multicenter study registered at https://ClinicalTrials.gov with the identifier number blinded in the journal article. The patients presented acute respiratory tract infection symptoms (eg., cough, dyspnea, fever) and the COVID-19 diagnosis was confirmed by RT-PCR and/or chest CT scan findings compatible with COVID-19. The participants were randomly grouped (no significant age and sex differences) in their first visit and received either a bottle containing 30 capsules of 25D or placebo. The same standard care (a combination of hydroxychloroquine and azithromycin) were given to all patients, and ceftriaxone for patients with pneumonia.

The study outlined six outcomes: 1, percentage of COVID-19 severity (mild, moderate, and severe) based on WHO criteria; 2, length of hospital stay counting from admission to discharge; 3, percentage of patients who need oxygen support; 4, rate of death due to COVID-19; 5, lymphocyte count and percentage; 6, serum 25D concentrations at three time points (baseline, 30 and 60 d after enrollment).

After supplementation, the circulating 25D concentrations significantly increased (30 d, 42.0 ng/mL; 60 d, 59.6 ng/mL; placebo 19.3–19.4 ng/mL), meeting one of the six outcomes outlined for the trial. However, there was no statistically significant difference in four of the other (clinical) outcomes (ICU admissions, need for ventilation, length of stay and rate of death) between the two groups.

#23: Hospitalization took a COVID-19 patient seven d (median) counting from the symptom onset, and most (85.9%) of the enrolled 85 Spanish severe COVID-19 patients showed bilateral pneumonia on x-rays. The main coexisting comorbidities were obesity (54.1%), hypertension (48.2%), dyslipidemia (36.5%), and diabetes (22.3%). The primary endpoint was the increase of 25D serum level ≥ 30 ng/mL after 14 d of the end of supplementation.

After D3 supplementation for 2 weeks in combination with the standard care in patients hospitalized with pneumonia due to COVID-19, the mean serum 25D levels increased from 14.8 ng/mL to 19.11 ng/mL in the low dose group (2000 IU/day), and to 29.22 ng/mL in the high dose group (10,000 IU/day) ($p < 0.0001$), which was still slightly shy from the primary endpoint (25D serum level ≥ 30 ng/mL). The length of hospital stay (one of the secondary endpoints) was not significantly different between both groups. However, beneficial effect (shorter stay at the hospital) was only observed in participants who developed ARDS and received high dose D3 supplementation when compared to the low dose group (8.0 vs. 29.2 d, $p = 0.0381$). The levels of haemoglobin and bilirubin in the high VD (10,000 IU/d) group were significantly higher ($p = 0.006$ and $p = 0.010$, respectively) at the end of supplementation, compared to low VD group.

#24 (55): 90 moderate to severe COVID-19 patients (45 each group) who were VD deficient were enrolled in this trial (SHADE-S, NCT04952857) to test the effect of high oral dose of D3 supplementation on SARS-CoV-2 clearance. The patients (13–14 d from symptom onset to recruitment) presented CT scan findings of the lung (bilateral multifocal ground-glass opacities ≥50%) and PaO2 /FIO2 lower than 200, an indication requiring invasive/non-invasive ventilation. The SARS-CoV-2 infection diagnoses were further confirmed by RT-qPCR. Upon admission at breakfast, patients received orally either placebo (medium-chain triglyceride oil) or a single high dose (0.6 million IU) of D3 (nano-droplet forms).

The primary outcome was the difference in SOFA score at d 7 between the two groups, and the secondary outcomes measured change in SOFA scores (at D 3, 10, 14), PaO2/FiO2 ratio, total duration of mechanical ventilation, all-cause mortality within 28 d of intervention, and the change in inflammatory markers (CRP, d-dimer, ferritin). The 25D levels were measured by in-house ECLIA using a kit. VD deficiency was defined as 25D level < 20 ng/mL, and severe VD deficiency <10 ng/mL.

Seven d after, the median 25D levels of the VD group raised from 12 ng/mL at baseline to 60 ng/mL whereas the placebo group changed from 13 ng/mL to 16 ng/mL; the SOFA score and PaO2 /FiO2 ratio of the VD group significantly improved ($p = 0.01$). D3 supplementation also significantly lowered CRP ($p = 0.003$) by d 7 and all-cause mortality at d 28 ($p = 0.046$); and interestingly a decrease in total calcium over time. Days on mechanical ventilation were lower in the VD group.

#25 (56): 50 patients were allocated through electronic randomization on the day of admission. They received no treatment or with calcitriol (1,25D) 0.5 μg daily for 14 d or hospital discharge (whichever was first). Four outcomes were prespecified: length of hospital stay, need for ICU admission, mortality, oxygen requirements, and readmission. 25D levels were not measured. Only a significant reduction in oxygen requirements in those who were supplemented.

Summary of, comment to and discussion on the 25 RCTs

Short summaries of the 25 RCTs

The 25 RCTs enrolled COVID-19 patients of diverging severity by local (regional or national) criteria, ranging from asymptomatic or mild (35), mild to moderate (36, 42, 54), moderate-to-severe (41, 48), severe (53, 57), to pneumonia (34, 43).

D3 (cholecalciferol) is the most often used form of supplemental VD (58), which is also the case in our reviewed RCTs (Table 1). 20 of the 25 RCTs supplemented with D3 manufactured by different companies, and the others used special VD supplements: two with 25D (34, 54), three with alphacalcidol [(41, 43, 56); the lower dose arm of RCT#8 used alphacalcidol too]. Comparing to its precursor D3, 25D supplementation is direct (no need to be converted in the liver) and more hydrophilic, absorbed better in the intestine (79% vs. 93%), therefore 3.2-fold more potent [calculated and reviewed in Quesada-Gomez and Bouillon (59)]. Conversely, 1 μg of D3 increased serum 25D by 1.5 ± 0.9 nmoL/L whereas 25D increased it by 4.8 ± 1.2 nmoL/L (59). Alphacalcidol (1α-D3) is a non-endogenous VD analogue, which is structurally different from D3 (Figure 1), and has longer pharmacological actions than D3 because of a negative feedback mechanism regulating the final activation step in the kidneys (60).

Regarding the dose and dosing strategy, there were three patterns. Over half of them (14/25) used 'same daily dose' for some time, ranging from a shortest period of 5 days (41) to the longest of 6 month (61); nine of them either used 'two high doses (RCT#19, each on day 1 and day 8)' or a single high dose (9 RCTs, #: 5, 8, 16–21 and 24); and three used 'intermittent higher to lower doses' (RCT#: 1, 6 and 14).

It was reported that high-dose bolus replacement may induce long-term expression of the catabolic enzyme 24-hydroxylase and fibroblast growth factor 23, both having VD inactivating effects (62), which might be one of the reasons why high-dose bolus of VD in some trial did not work (51, 52). Contrarily, a 3-month long RCT study comparing efficacy of daily, weekly and monthly administration of D3 demonstrated equal efficacy (63).

For ethical reasons, some RCTs had not set up placebo group (eight RCTs, #: 1, 7, 10, 12, 13, 17, 19, and 21) or lacked both control/placebo groups (six RCTs, #: 3–5, 8, 11, and 23). Some were single-blinded (RCT#: 23 and 25) or double blinded (seven RCTs, #: 1, 4, 14, 16, 18, 22, 24), and some were open-labeled (nine RCTs, #: 1, 5, 10, 11, 15, 17, 19, 21, and 26).

Among the trials with 25D serum concentration data available, only two RCTs had optimal (≥30 ng/mL) levels at baseline (42, 50), the others were in VD deficit status (ranging between insufficiency to severely deficiency). After supplementation by varying strategies, ten RCTs had endline 25D serum concentrations slightly shy from [De Niet et al. (39), Cannata-Andía et al. (49), and Torres et al. (57)] or beyond the optimal level (35, 38, 42, 48, 50, 54, 61).

Comments to and discussion on the 25 RCTs

Considering the nearly hopeless situation the COVID-19 pandemic had put the whole world in, it is understandable that the 25 RCTs we here focused on had set various and sometimes very different prespecified primary and secondary outcomes.

Judging from a clinical perspective, one would say some RCTs in this series of trials fully or partly succeeded (e.g., RCTs #1–6, 8–10, 23, 24), and some failed (RCTs #17–22). Following are a brief comment to and comparison of some of the trials and, discussion of the possible reasons of their success and failure.

TABLE 1 Some features of the reviewed RCTs.

RCT	Participants		Mean age (years)		VD type	Dose (IU) (cumulative dose)	How/ when	How long	VD level (ng/ml, some changed from nmol/L)		Country	PMID (ref)
	VD	C/P	VD	C/P					pre	after		
#1	50	26	53	52.8	25D	532 mcg/d 1, 3, 7; then 266 mcg /w	See left box	UD or ICU	NA	NA	Spain	32871238 (34)
#2	16	24	50	47.5	D3	60,000 (4.8E5 to 8.2E5)	Daily/1 w; weekly	1 or 2 w	D 8.6 P 9.5	D **51.7** P 15.2	India	33184146 (35)
#3	36/33	0	46.3/53.5	NA	D3	1,000 or 5,000 (1.4E4 or 7E4)	Daily	2 w	H 21.4 L 25.2	H 25 L 21.9	Saudi Arabia	34202578 (36)
#4	161	160	36	39	D3	4,000 (1.2E5)	Daily	1 m	D 18.4 P 16.5	D 26.1 P 19.3	Mexico	35487792 (37)
#5	127/127	0	87/89	NA	D3	50,000 or 400,000 (5E4 or 4E5)	**Once** only	NA	H 20.8 S 17.2	H **60.4** S 25.6	Netherlands	35639792 (38)
#6	26	24	63.2	68.7	D3	25,000 d: 1–4, 8, 15, 22, 29 (2E5)	See left box	8 d/m	D 17.9 P 16.9	D 29.9 P 16.9	Belgium	35893907 (39)
#7	20	25	10.7	14	D3	1,000 or 2,000 (1.4E4 or 2.8E4)	Daily	2 w	D 13.8 C 11.4	NA NA	Mexico	35958172 (40)
#8	58/58	0	66.1/65.7	NA	1 − α 25D/ D3	25D: 1mcg/ d or D3:200,000 (**IM**) (2E5)	25D: daily; D3: **once**	5 d	NA	NA	Egypt	36295519 (41)
#9	65	69	42.1	43.8	ERC	300 mcg /d 1–3 & 60 mcg /d 4–27	Daily	27 d	D **37.7** P **37.1**	D **81.8** P **34.8**	USA	36529089 (42)
#10	147	147	47.9	53.7	1 − α 25D	2 mcg	Daily	UD	D 22.5 C 20.8	NA NA	Thailand	38383361 (43)
#11	75	162	63.3	63.3	D3	5,000 (7E4)	Daily	2 w	9.64	NA NA	Austria	34976511 (44)
#12	25	25	41.4	46.4	D3	360 (0.5E4)	Daily	2 w	NA	NA	Pakistan	35747751 (45)
#13	30	30	50	26.0	D3	2,000 (2.8E4)	Daily	2 w	NA	NA	Egypt	36425571 (46)
#14	90	91	NA	NA	D3	180,000, then 2,000/d (2.92E5)	**Once**, then daily	8 w	D 18.2 P 23.5	D 26.7 P 22	India	37560461 (47)
#15	1,550 1,550	3,100	?	60.8	D3	800 or 3,200 (1.44E5 or 5.76E5)	Daily	6 m	H 16.4 L 16.6 C NA	H **41.2** L **31.6** C 21.5	Brazil	36215226 (61)
#16	120	120	/	/	D3	200,000 (2E5)	**Once** only	NA	D 21.2 P 20.6	D **44.4** P 19.8	Brazil	33595634 (48)
#17	274	269	59.0	57.0	D3	100,000 (1E5)	**Once** only	NA	D 17 C 16.1	D 29 C 16.4	Spain	35177066 (49)
#18	115	105	59.8	58.3	D3	500,000 (5E5)	**Once** only	NA	D **32.5** P **30.5**	D **102** P **30**.0	Argentina	35622854 (50)
#19	56	54	58	64	D3	50,000 (5E4)	d1 d8	NA	D 16.4 P 13.9	D 22.8 P 10.6	Russia	35807783 (51)
#20	57	60	43	41	D3	200,000 (2E5)	**Once** only	NA	NA	NA	Tunisia	36803273 (52)
#21	75	77	65	65.5	D3	10,000 (1E4)	Daily	≧2 w	D 10.1 C 10.9	D 22.5 NA	Croatia	36904232 (53)

(Continued)

TABLE 1 (Continued)

RCT	Participants		Mean age (years)		VD type	Dose (IU) (cumulative dose)	How/ when	How long	VD level (ng/ml, some changed from nmol/L)		Country	PMID (ref)
	VD	C/P	VD	C/P					pre	after		
#22	53	53	50	49	25D	25 mcg	Daily	2 m	D 19 P 18	D **59.6** P 19.4	Iran	34653608 (54)
#23	41/44	0	67/65.3	NA	D3	2,000 or 1,0000 (2.8E4 or 1E5)	Daily	2 w	H 15.3 L 14.3	H 29 L 19	Spain	35468580 (57)
#24	45	45	51	46	D3	600,000 (6E5)	**Once** only	NA	D 12 P 13	D **60** P 4	India	38291897 (55)
#25	25	25	69	64	1,25D	0.5 mcg	Daily	2w	NA	NA	USA	34508882 (56)

25D, 25-hydroxycholecalciferol, calcifediol; c, control; C/P, Control/Placebo; d, day; (V)D, vitamin D; D3, cholecalciferol, calcidiol; ERC, extended-release calcifediol; H, high dose; IM, intramuscular injection; L, low dose; m, month; MCG, microgram; NA, not available/applicable; once (only), a single dose; p, placebo; RCT,; S, standard dose; w, week(ly); y, year; UD, until discharge; ICU, ICU admission; numbers after one decimal are rounded up; 1E4 = 10,000; 10 mcg of calcifediol = 2000 IU of D3 https://vitamored.com/products/vitamored-vegan-vitamind3-calcifediol. Bold values indicating above the sufficiency level.

No matter successful or not, some RCTs lacked a key information, the 25D levels at baseline and/or after supplementation of some/all groups, which hinders comparison analysis between baseline and endline VD status (sufficient, insufficient, or deficient) of the tested subjects that is one of the central points of VD supplementation. That is the case for eleven out of the 25 trials (detailed in Table 1); specifically, six RCTs (#: 1, 8, 12, 13, 20, 25) lacked the full set data (25D values at baseline and after supplementation), and five RCTs (#: 6, 7, 10, 11, 21) had incomplete information. So, the discussion will basically leave some of these eleven RCTs out due to lack of data.

One thing to bear in mind is that trial success is relatively speaking and also related to the severity of the infected participants, it will be hard to imagine the outcome if RCT#2 and RCT#3 enrolled severe COVID patients instead of mild to moderate COVID-19 patients (35, 36).

Technically speaking, RCT#2 succeeded in meeting its prespecified outcome (35). Compared to placebo group, more people in the supplementation group became SARS-CoV-2 RNA negative ($p < 0.018$), in contrast to the failure of RCT#20 in this aspect (52). The main difference in supplementing D3 to boost the VD level is that RCT#20 used a single high dose (200,000 IU) without knowing baseline and endline VD status, while RCT#2 used 60,000 IU daily per oral to make the 25D level reach >50 ng/mL at day 7; if not, continued for another week. The supplemented patients in RCT#2 received at least twice the total dose compared to the single high dose only in RCT#20. Of note, RNA test from positive to negative only indicates the decrease of the potential contagiousness, not exactly equal to clinical improvement(s).

RCT#3, together with the other five trials (RCTs #5, 16–18, 22) were carried out at multiple centers. Two met (RCT# 3 and 5) but the rest three failed to meet their respective outcome(s). There are several interesting with some baffling findings from these six multicenter trials. As of VD types for supplementation, only RCT#22 used daily 25D for 30–60 d (54), and the other five used D3 by differing strategies. Strategy-wise for the five trials using D3, RCT#3 used daily 1,000 or 5,000 IU for 2 weeks (36) while the others used single high doses: 50,000 or 400,000 IU in RCT#5, 100,000 IU, 20,0000 IU, or 500,000 IU, respectively, for RCTs #17, #16 and #18.

Comparing to failure of their respective low dose group (1,000 IU daily for 2 weeks and 50,000 single dose) in RCT#3 and RCT#5, both of their high dose groups (5,000 IU daily for 2 weeks or 400,000 IU single dose) succeeded to achieve their respective clinical outcomes. Comparing to the high dose groups of RCT#3 and RCT#5 and their endline mean 25D levels (25 ng/mL vs. 60 ng/mL), the other four trials using either single high dose or daily VD achieved comparable or even higher endline mean 25D levels (44.4 ng/mL in RCT#16, 29 ng/mL in RCT#17, 102 ng/mL in RCT#18, and 42 ng/mL in RCT#22). The baffling question is why the other four trials failed to meet their respective outcomes while they succeeded to significantly correct the VD level? There may be other rational hypotheses, but one of the possibilities is that the endline 25D serum concentration upon VD supplementation is not an indicator guaranteeing success in counteracting a COVID-19 infection.

There were several differences among these six trials besides differences of dose, dosing strategy and serum 25D levels. They enrolled COVID-19 patients of different features: mild to moderate severity of patients in RCT#3 and RCT#16–18; while the high dose group in RCT#3 was younger and less obese (the outcome difference remains significant after adjusting for age, sex, baseline BMI, and D-dimer); RCT#16 had several nationalities with varying baseline 25D levels (ng/mL; Guatemala 24.1, Chile 19.5, Argentina 16.0, Spain 13.4); more than half were white and over 30% mixed ethnicity in RCT#17 while it took 10 days from symptom onset to enrollment; and patients in RCT#18 had risk factors for disease progression; RCT#5 had old patients (median age 88) who was diagnosed by RT-PCR or chest CT scan within 72 h in (also the latest time VD supplementation started); and the severity of patients in RCT#22 was not specified and the trial was terminated after reviewing of first stage results. All the above distinct baseline characteristics can theoretically affect the outcomes. Genetic factors influence not only baseline serum 25D concentration (64), but also the response to VD supplementation (65). Similarly, some other RCTs also had not specified the criteria of defining the severity of the infection (23, 35, 41, 43, 44, 47, 49, 51, 52), some (34, 46) used well defined criteria with a traceable reference [such as (66)], the CURB65 severity scale (67), and some employed criteria self-defined (42, 45, 48, 50) or outlined by their local health departments (36, 40). For example,

corticosteroids, used in some RCTs (41, 43), could confound the outcomes of VD supplementation on COVID-19 infection (68).

Moreover, serum 25D was measured differently, which is also a key concern expressed in a recent review (58). RCT#3 used a fully automated CLIA analyzer, RCT#5, RCT#16 and RCT#18 used CLIA, RCT#17 used ELIA or CLIA, and RCT#22 used HPLC (54). The differences between different assays are huge, and even the same type assay but using different protocols and/or reagents, which all contribute to variations of the serum 25D values.

Two RCTs were aimed to investigate whether VD supplementation could prevent COVID-19. RCT#4 was successful in significantly lowering SARS-CoV-2 infection rate in frontline healthcare workers (37). By contrast, RCT#15 concluded that VD supplementation was unable to reduce the risk of COVID-19 (61). Here the 25D puzzle came again. The '4,000 IU VD daily for 30 d' strategy in RCT#4 had changed the 25D level from 18.4 ng/mL to 26.1 ng/mL (below the sufficient level) but it worked to lower the COVID-19 (37). On the contrary, daily supplementation of VD (800 IU or 3,200 IU) for 6 month raised the VD level from about 16 ng/mL to 31.6 ng/mL and 41.2 ng/mL (61), both above the sufficient level set by the Society (19), but both failed to reduce risk of COVID-19.

Besides the dose/dosing difference, there were other differences between these two trials. RCT#4 was a relatively small scale trial, with less than 200 Mexican healthcare workers (median age 36.5), and some of them might unknowingly have exposed to COVID-19 due to their occupation risk, become immunized but remained asymptomatic, because only RT-PCR tests were taken at baseline (37). Moreover, the VD group was significantly younger (36 vs. 39, $p = 0.019$); the concentration of 25D was determined using a Waters ACQUITYH UPLC. RCT#15 was the largest among all trials, with more than 6,000 participants of median age of 60.2 and about 95% were white (61). Of note, concentrations of both serum 25D ($25D_3$ and $25D_2$) were measured by LC-TMS using dried blood spot eluates (61).

RCT#6 used an intermittent dosing strategy (25,000 IU per day for the first 4 d, then the same dose per week for up to 6 weeks) and raised the serum 25D from 17.9 to 29.9 ng/mL, slightly shy from the sufficient level. The supplementation decreased significantly the length of hospital stay (39).

RCT#7 was the only one targeting pediatric COVID-19 patients, but it was prematurely ended due to ethical reason (40), and apparently such trial is warranted in the future. Although still controversial, the general consensus from studies of different natures (sectional, observational, cohort) suggests VD supplementation is essential to those whose VD levels are sub-optimal but the usage should be carefully monitored to prevent overdosing (58).

RCT#8 have two interesting features. One is that in the same trial the investigators decided to use two different VD supplements by two different routes for supplementation. The low dose group orally took 25D (alfacalcidol; 1 mcg/day) for 5 days, and the high dose group received an extra single intramuscular injection of D3 (cholecalciferol; 200,000 IU) upon enrollment. The other interesting feature is that the investigators knew the virus causing these infections was a D614G mutant strain which was prevalent in Egypt at the trial period and associated with higher viral loads and probably with enhanced transmissibility compared to other variants (66). Among the 25 RCTs reviewed, this is the only study reported the causal virus information of the trial. It is well-known that SARS-CoV-2 variants differ in their virulence and epidemiology, causing COVID-19 diseases of varying severity, which is also relating to evaluation of therapy and prevention trials. Unfortunately, it lacked serum 25D data both at the baseline and after supplementation (41), which made it impossible to compare their VD correction effects of the different supplementation routes (oral vs. intramuscular) and types of VD supplements used (D3 vs. 25D).

RCT#9 has two unique features among all. It is the only trial using extended-release calcifediol (ERC) for supplementation, and one of the only two trials that enrolled participants whose baseline VD were at the sufficient levels (42, 50). ERC has a different pharmacokinetics than the conventional 25D product (used in RCT#22) by releasing 25D gradually over a period of 12 h (69).

RCT#10 demonstrated that VD supplementation only worked for a subset of patients (those require supplemental oxygen or high-dose corticosteroid therapy or have high CRP >30 mg/L), but not to all enrolled patients, which helps understand the conflicting results between all trials (43).

RCTs #11–14 were trials using VD as one of the combo components, they were either designed without an VD alone group or VD alone did not work at all (44–47). In the future, similar of such combo trials need better designed to have a VD alone group if wanting to see the independent effect(s) of VD supplementation.

RCT#24 and RCT#2 were the only two RCTs carried out in the same hospital (Nehru hospital of north India) among the 25 trials reviewed. RCT#2 (SHADE; NCT04459247) started 1 year earlier (2020-06-15) than RCT#24 (SHADE-S; NCT04952857; 2021-08-01). One of the difficulties when comparing single center RCTs is that they were carried out in different medical institutions using different brands of similar machines, reagents, and protocols to run assays, which creates heterogeneous results. In this sense, it is interesting to compare these two trials held in the same institution using very similar if not identical logistics (machines, reagents, and protocols). The only major differences between these two trials would be on the patients and the dose/dosing. Still, there are similarities and differences between them.

Similarities. Both trials were successful and of the same design (randomized, double-blind, placebo-controlled) and comparable scale (less than 100 patients), enrolled vitamin D-deficient patients, used the similar if not identical instruments, reagents and protocols to measure 25D, inflammatory markers and so on (35, 55).

Major differences. The SHADE study enrolled asymptomatic or mildly symptomatic patients (thus, different baseline values of their demographic parameters, inflammatory markers and so on), gave the intervention group daily 60,000 IU of D3 for 7 d (or 14 d if 25D not >50 ng/mL), looked for SARS-CoV-2 RNA negativity as the primary outcome (35). By contrast, the SHADE-S trial enrolled severe COVID-19 patients and gave the intervention group a single high-dose (0.6 million IU) of D3, and looked for SOFA score at D 7 as the primary outcome (55).

Conclusion

Together, the general conclusion from these studies is that VD insufficiency/deficiency is highly related to COVID-19 infection, its severity and mortality, but data of the effect on clinical benefit from VD supplementation is conflicting, further RCT study is surely needed.

One key but puzzling observation after carefully reviewing these 25 RCTs is that the endline serum 25D concentration, although a good indicator of the VD supplementation effect on correcting VD insufficiency/deficiency, it is not reliable to predict that VD sufficiency

after supplementation is a guarantee of clinical improvement of COVID-19. There are 7 RCTs reviewed above that had endline serum 25D concentration at or above the optimal level (#: 2, 5, 9, 15, 16, 18, 22; the exact ng/mL values in Table 1; optimal level ≥ 30 ng/mL) but only three reached its trial outcome (RCTs, #2, 5, 9) and the other four failed, despite having the VD deficit of the patients corrected. By contrast, the supplementation in three RCTs (#3, 4, 6) failed to correct the VD insufficiency/deficiency but succeeded in improving the clinical outcome(s). Apparently, the scientific community need to work out a (set of) biomarker(s) that can be used as a correlate of the effect of VD supplementation on protection (prophylactic), treatment (therapeutic) or both.

Although it is a long way to go, there are already some pioneering work that has been done. Among others, calprotectin (70, 71), endocan (72), growth differentiation factor 15 (GDF15) (73), inflammatory cytokines (IL1 and IL6) (74), miRNAs (75), neopterin (76), soluble suppressor of tumorigenicity 2 (sST2) (77), and T cell immunoglobulin and mucin domain containing protein 3 (Tim) (78) have been reviewed having the potential as biomarkers for COVID-19 severity.

Author contributions

LH: Writing – original draft, Writing – review & editing, Conceptualization. ZS: Data curation, Investigation, Writing – review & editing. CL: Data curation, Investigation, Writing – review & editing. SW: Data curation, Investigation, Writing – review & editing. CG: Data curation, Investigation, Writing – review & editing. X-HL: Writing – original draft, Writing – review & editing, Conceptualization. ZZ: Writing – original draft, Writing – review & editing, Conceptualization.

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Conflict of interest

CG was employed by Shaoxing BWK Biotechnology Co., Ltd. ZZ was employed by Hebei Huiji Technology Co., Ltd. X-HL was employed by Shenzhen Boya Gene Technology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1461485/full#supplementary-material

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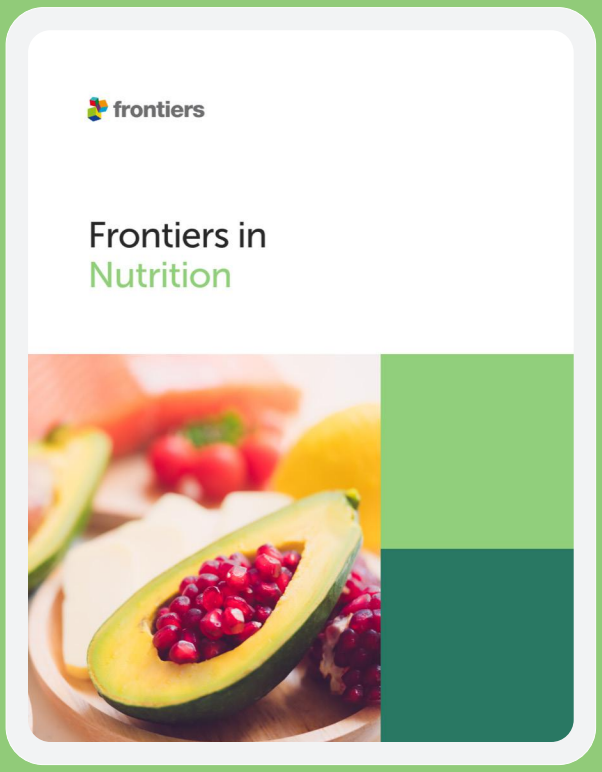

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