LMJ LINK MEDICAL JOURNAL OF HEALTH AND COMMUNITY RESEARCH

Crossref DOI: https://doi.org/10.61919/kk8apw75

*Original Article*

# The Association Between Serum Vitamin D Status and Knee Joint Space Width in Adults with Early Osteoarthritis - A Cross-Sectional Study

**Namra Urooj[1], Sumera Kanwal[2], Malak Mustaqeem Khan[3], Saima Ashraf[4], Nauman Akhtar[5], Aqil ur Rehman[6], Muhammad Faizan Ali[7]**

[1] Physical Therapist, Mukabbir University, Gujrat, Pakistan
[2] Assistant Professor, Faculty of Health and Medical Sciences, Metropolitan University, Karachi, Pakistan
[3] Postgraduate Resident, Department of Orthopaedic Surgery, CPSP, Lady Reading Hospital, Peshawar, Pakistan
[4] Senior Lecturer, Department of Veterinary Anatomy, School of Veterinary Medicine, IMU University, Kuala Lumpur, Malaysia
[5] Medical Officer, PAF Hospital, Faisal, Karachi, Pakistan
[6] Assistant Scientific Officer, Food Science Research Institute, National Agricultural Research Centre, Islamabad, Pakistan
[7] MBBS Graduate, Aziz Fatima Medical and Dental College, Faisalabad, Pakistan

***Corresponding author: Namra Urooj, namra.urooj@yahoo.com**

## ABSTRACT

Background: Knee osteoarthritis (OA) is a progressive degenerative joint disease in which early cartilage loss determines long-term functional trajectory. Vitamin D, beyond its classical skeletal role, modulates chondrocyte metabolism and joint inflammatory signaling through vitamin D receptor pathways; however, its cross-sectional association with radiographic cartilage preservation in early knee OA remains incompletely characterized. Objective: To investigate the association between serum 25-hydroxyvitamin D [25(OH)D] sufficiency and medial tibiofemoral joint space width (JSW) as a radiographic marker of cartilage integrity in adults with early knee osteoarthritis. Methods: A cross-sectional study was conducted over eight months at a tertiary care hospital in Lahore, Pakistan, enrolling 135 adults aged 40–65 years with Kellgren–Lawrence grade I–II knee OA. Serum 25(OH)D was measured by chemiluminescent immunoassay and categorized as deficient (<20 ng/mL), insufficient (20–29 ng/mL), or sufficient (≥30 ng/mL) per Endocrine Society guidelines. Standardized weight-bearing JSW was measured digitally by two blinded musculoskeletal radiologists (ICC = 0.91). WOMAC pain and function subscales provided secondary clinical outcomes. ANOVA with Tukey post-hoc testing, Pearson correlation with 95% CIs, and multivariable linear regression adjusting for age, sex, BMI, and symptom duration were applied. Results: Mean serum 25(OH)D was 26.7 ± 8.4 ng/mL. Sufficient participants demonstrated significantly greater JSW than deficient individuals (3.89 ± 0.57 mm vs. 3.11 ± 0.54 mm; mean difference 0.78 mm, 95% CI 0.51–1.05, $p < 0.001$). Serum 25(OH)D correlated positively with JSW ($r = 0.41$, 95% CI 0.26–0.54, $p < 0.001$) and inversely with WOMAC pain ($r = -0.36$, $p = 0.002$) and function ($r = -0.33$, $p = 0.004$). Associations remained significant after confounder adjustment ($\beta = 0.023$ mm/ng/mL, 95% CI 0.011–0.035, $p < 0.001$). Conclusion: Vitamin D sufficiency was independently associated with greater radiographic joint space width and reduced symptomatic burden in early knee OA, supporting its evaluation as a modifiable nutritional target in early OA prevention. Keywords: 25-Hydroxyvitamin D; joint space width; Kellgren–Lawrence grade; knee osteoarthritis; nutritional status; radiography; WOMAC

**"Cite this Article"** | **Received**: 23 October 2025; **Accepted**: 26 December 2025; **Published**: 31 December 2025.

**Author Contributions:** Concept: NU; Design: NU, SK; Data collection: MMK, NA; Analysis: AR, MFA; Drafting: NU, SK, SA, MNR, KBK, LM. **Ethical Approval**: Mukabbir University, Gujrat, Pakistan. **Informed Consent**: Written informed consent was obtained from all participants; **Conflict of Interest**: The authors declare no conflict of interest; **Funding**: No external funding; **Data Availability**: Available from the corresponding author on reasonable request; **Acknowledgments**: N/A.

## INTRODUCTION

Osteoarthritis (OA) represents one of the most prevalent chronic musculoskeletal conditions globally, affecting an estimated 528 million individuals and constituting a leading cause of pain, functional limitation, and reduced quality of life across aging populations (1). Among all affected joints, the knee is disproportionately impacted, both in prevalence and in the severity of functional consequences attributed to progressive articular cartilage degradation (2). In the early stages of knee OA, classified radiographically as Kellgren–Lawrence (KL) grades I and II, subchondral and chondral changes may remain clinically subtle yet structurally significant; joint space width (JSW) measured on weight-bearing radiographs serves as the established radiographic surrogate for medial tibiofemoral cartilage integrity

during this critical window (3). The pathogenesis of knee OA is multifactorial, implicating biomechanical overloading, systemic inflammatory cascades, metabolic dysregulation, and nutritional insufficiency as interdependent contributors to cartilage matrix deterioration (4). Among nutritional factors, vitamin D, a secosteroid hormone classically recognized for its regulation of calcium homeostasis and skeletal mineralization, has attracted increasing scientific attention for its potential role in maintaining articular cartilage architecture beyond its established skeletal functions (5).

The biological plausibility for a vitamin D–cartilage axis is well supported by cellular and pre-clinical evidence. Vitamin D receptors (VDRs) are constitutively expressed in chondrocytes and subchondral osteoblasts, where the active metabolite 1,25-dihydroxyvitamin D modulates chondrocyte proliferation, differentiation, and extracellular matrix synthesis, including type II collagen and aggrecan production (6). Deficiency in circulating 25-hydroxyvitamin D [25(OH)D], the principal storage form measured clinically, has been associated with dysregulated matrix metalloproteinase activity, impaired subchondral bone remodeling, and an amplified pro-inflammatory microenvironment within the joint, characterized by elevated interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (7, 8). Experimental models further demonstrate that $1,25(OH)_2D$ supplementation attenuates cartilage degradation and reduces synovial inflammatory mediator release, reinforcing the mechanistic rationale for investigating vitamin D status as a modifiable determinant of joint structural preservation (9). These pathways collectively suggest that serum 25(OH)D insufficiency may accelerate the articular cartilage loss that defines early OA progression (10).

Observational and epidemiological data have provided substantial, if not entirely consistent, support for this hypothesis. A large retrospective analysis of 3,424 subjects in Turkey found a significant inverse relationship between serum vitamin D levels and the prevalence of radiographically confirmed knee OA, with lower 25(OH)D concentrations independently associated with greater odds of disease presence across age and sex strata (11). A dedicated cross-sectional investigation of adults with early knee OA demonstrated that vitamin D insufficiency was associated with impaired physical function and heightened pain scores, even at KL grades I–II, suggesting that structural and symptomatic consequences of low vitamin D status may manifest before advanced degeneration occurs (4). A retrospective study examining postmenopausal women further reported that lower serum 25(OH)D correlated with reduced bone mineral density and more severe radiographic OA grading in the knee, underscoring the potential relevance of vitamin D status at the bone–cartilage interface (12). Meta-analytic synthesis has similarly indicated that vitamin D deficiency is associated with worsening symptomatic and possibly structural OA outcomes, although effect heterogeneity across studies has prevented definitive conclusions regarding the magnitude of the structural association (13, 14).

Nonetheless, the literature is complicated by interventional trials that have not consistently translated observational associations into therapeutic benefit. In a five-year randomized controlled trial examining the long-term effects of vitamin D supplementation in knee OA, supplementation failed to significantly reduce medial joint space narrowing or improve pain and function outcomes compared with placebo, even after sustained correction of baseline deficiency (15). Similarly, a landmark randomized placebo-controlled trial published in JAMA demonstrated that two years of vitamin $D_3$ supplementation did not reduce knee pain scores or attenuate cartilage volume loss measured by MRI, regardless of baseline 25(OH)D levels (16). These findings are not necessarily contradictory to observational evidence; rather, they suggest that vitamin D may function as a systemic marker of joint health risk rather than a pharmacological target capable of reversing established cartilage degeneration when administered in isolation. The distinction between habitual sufficiency, reflecting long-term nutritional adequacy and its downstream effects on joint metabolism, and short-term supplementation as a rescue strategy is biologically and clinically important and has not been adequately explored in early, pre-erosive OA (17). Additionally, recent Mendelian randomization analyses have not established unambiguous causal directionality between genetically predicted 25(OH)D and OA risk, further supporting the interpretation that the observed clinical associations are influenced by multiple interacting exposures (18).

Geographic, demographic, and methodological variability further complicate the synthesis of this evidence. Studies conducted in sub-Saharan African populations have reported no significant association between serum vitamin D levels and OA-related pain or physical function, implying that lifestyle factors, sun exposure patterns, genetic variation in VDR polymorphisms, and dietary habits may substantially modify the vitamin D–OA relationship across populations (19). In South Asian and Middle Eastern cohorts, vitamin D deficiency is endemic and co-exists with metabolic conditions that independently influence musculoskeletal health, creating a complex confounding environment that demands population-specific investigation (20). Furthermore, most published cross-sectional studies examining this relationship have relied on either radiographic or clinical endpoints in isolation, without simultaneously integrating biochemical assessment of vitamin D status with standardized radiographic JSW measurement and validated functional outcomes within a single early-OA cohort (21, 22). This methodological fragmentation limits the interpretability of existing findings and prevents a comprehensive picture of how vitamin D status relates to both the structural and symptomatic dimensions of early knee OA concurrently.

A critical knowledge gap therefore persists: despite the mechanistic plausibility and partial observational support for vitamin D's role in cartilage preservation, no study conducted in a South Asian adult population with radiographically confirmed early knee OA has simultaneously characterized the cross-sectional association between serum 25(OH)D sufficiency and standardized JSW as a radiographic marker of medial tibiofemoral cartilage integrity, while also capturing validated pain and functional outcomes. Addressing this gap is clinically meaningful because early OA represents the therapeutic window during which modifiable nutritional factors may exert their greatest preventive influence on structural progression. The present study therefore aimed to investigate the association between serum vitamin D status and knee joint space width in adults with early osteoarthritis, and to determine whether vitamin D sufficiency is independently correlated with radiographic cartilage preservation after adjustment for key demographic and anthropometric confounders.

## MATERIAL AND METHODS

This cross-sectional observational study was conducted over eight consecutive months at a tertiary care hospital in Lahore, Pakistan. The study protocol was reviewed and approved by the Institutional Review Board of the recruiting institution (Approval Reference No.: [IRB-XXX/20XX]; date of approval: [month, year]), and all procedures were carried out in full conformity with the ethical principles set forth in the 2013 Declaration of Helsinki. Written informed consent was obtained from every participant before enrollment, following a structured briefing in which the study's objectives, procedures, potential risks, and voluntary nature of participation were explained in the participant's preferred language. All data were collected, stored, and reported in anonymized form, with participant confidentiality maintained throughout.

The study population comprised adults attending the rheumatology and orthopaedic outpatient clinics with clinical and radiographic evidence of early knee OA. Eligible participants were adults aged 40 to 65 years presenting with knee pain persisting for more than three months and demonstrating radiographic features consistent with KL grades I or II on standardized weight-bearing anteroposterior knee radiographs. Participants were enrolled using consecutive sampling, whereby every patient attending the outpatient clinics during the study period who satisfied the eligibility criteria was invited to participate until the target sample size was reached. This approach ensured systematic recruitment while minimizing selection bias inherent in non-consecutive or convenience-based designs. Participants were excluded if they had a documented history of metabolic bone disease (rickets, osteomalacia, or Paget's disease), rheumatoid arthritis, microcrystalline arthropathy (gout or pseudogout), inflammatory bowel disease, chronic kidney disease (eGFR < 60 mL/min/1.73 $m^2$), hepatic insufficiency, or endocrine disorders known to influence bone or mineral metabolism, including primary hyperparathyroidism and thyroid dysfunction. Individuals who had received oral or intramuscular vitamin D supplementation or calcium

supplementation within the preceding three months were excluded; this washout period was selected based on the pharmacokinetic profile of 25(OH)D, which has a circulatory half-life of approximately two to three weeks, such that a 12-week interval is sufficient to allow supplementation-derived elevations to dissipate and allow serum concentrations to reflect habitual endogenous and dietary status (23). Participants with a history of ipsilateral knee surgery, intra-articular corticosteroid or hyaluronic acid injection within the preceding six months, or radiographically evident traumatic knee deformity were also excluded, as these conditions independently alter articular cartilage integrity and JSW independent of nutritional status.

The required sample size was determined a priori using the formula for correlation-based observational studies: $n = [(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (1 - r^2)] / r^2$, where $Z_{1-\alpha/2} = 1.96$ (two-tailed $\alpha = 0.05$) and $Z_{1-\beta} = 0.842$ (power = 80%). The anticipated correlation coefficient of $r = 0.25$ between serum 25(OH)D and knee JSW was derived from a population-based cross-sectional study in postmenopausal women that quantified the relationship between serum vitamin D levels and radiographic OA severity at the knee joint (24). This estimate was selected as a conservative projection applicable to a mixed-sex early OA cohort with moderate vitamin D insufficiency. The calculation yielded a minimum required sample of 122 participants; accounting for an anticipated 10% non-response and data-exclusion rate attributable to inadequate radiograph quality or incomplete laboratory results, a total of 135 participants were recruited to ensure adequate power.

All eligible participants underwent standardized clinical and radiographic assessment at a single study visit. Demographic data, including age, sex, body mass index (BMI, calculated as weight in kg divided by height in $m^2$), occupational category, and symptom duration in months, were recorded by trained research staff using a structured proforma. BMI was measured using a calibrated digital stadiometer and weighing scale, with participants assessed barefoot and in light clothing. Radiographic evaluation was performed using standardized weight-bearing anteroposterior and lateral knee radiographs obtained in full extension with the patella centred over the femoral condyles and the central beam directed 10° caudally; this protocol was uniformly applied to all 135 participants to ensure positional consistency, as JSW is known to vary by up to 1–2 mm with changes in knee flexion angle. Radiographs were evaluated independently by two fellowship-trained musculoskeletal radiologists who were blinded to participants' serum vitamin D status and clinical scores. JSW was quantified at the narrowest point of the medial tibiofemoral compartment using calibrated digital radiographic software, with measurements expressed in millimetres to the nearest 0.1 mm. The mean of the two independent readings was used as the final JSW value for each participant. Discrepancies exceeding 0.2 mm between observers were resolved by consensus review of the original image by both radiologists. Inter- and intra-observer reliability were quantified using the intraclass correlation coefficient (ICC, two-way mixed-effects model, absolute agreement), and both exceeded the pre-specified acceptability threshold of 0.90.

Venous blood samples of 5 mL were collected from each participant between 08:00 and 10:00 hours following an overnight fast of at least eight hours to minimize diurnal variation in biochemical analytes. Serum 25(OH)D concentration was measured using a validated chemiluminescent immunoassay (CLIA) on a fully automated immunoanalysis platform, with internal and external quality control standards applied at each analytical run to ensure inter-assay precision and accuracy. In accordance with the Endocrine Society Clinical Practice Guidelines, participants were categorized as vitamin D deficient (serum 25(OH)D < 20 ng/mL), insufficient (20–29 ng/mL), or sufficient (≥ 30 ng/mL) (25). Additional biochemical parameters, serum total calcium, inorganic phosphate, and alkaline phosphatase, were measured simultaneously to exclude subclinical metabolic bone disease that could independently affect chondral metabolism; participants with any value exceeding two standard deviations from the age- and sex-specific reference range were reviewed and, if metabolic pathology was suspected, were excluded post hoc.

Participant-reported pain and physical function were assessed using the validated Arabic and Urdu versions of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), which has been psychometrically validated for administration in South Asian adult populations with knee OA (26). The WOMAC pain subscale (five items, score range 0–20) and physical function subscale (17 items, score range 0–68) were scored using the standard Likert format, with higher scores indicating greater symptom burden. These instruments served as secondary outcome measures reflecting symptomatic status and were correlated with the primary radiographic outcome (JSW) to provide an integrated clinical–structural characterization of the study cohort.

All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD) and categorical variables as absolute frequencies and percentages. The Shapiro–Wilk test was used to verify the normality of continuous variable distributions prior to parametric testing. No participants had missing data on the primary radiographic or biochemical outcome; two participants had incomplete WOMAC subscale responses, and these items were imputed using the subscale mean for that participant, consistent with the WOMAC scoring manual. Between-group comparisons of mean JSW and WOMAC scores across the three vitamin D status categories were performed using one-way analysis of variance (ANOVA), with Tukey's honestly significant difference (HSD) post-hoc test applied to all three pairwise comparisons to control the family-wise Type I error rate. The strength and direction of the linear association between serum 25(OH)D and JSW, WOMAC pain, and WOMAC function were quantified using the Pearson correlation coefficient (r) with corresponding 95% confidence intervals (CIs). Multivariable linear regression analysis was conducted with JSW as the continuous dependent variable and serum 25(OH)D as the primary predictor of interest, adjusting for age, sex, BMI, and symptom duration as pre-specified confounders selected on the basis of biological plausibility and evidence from prior literature. Prior to constructing the regression model, multicollinearity among predictor variables was assessed using the variance inflation factor (VIF); all VIF values fell below 3.0, confirming the absence of problematic collinearity. Model assumptions, including homoscedasticity of residuals and normality of the error distribution, were evaluated graphically using standardized residual plots and a normal probability plot, respectively, and no meaningful violations were identified. Standardized and unstandardized regression coefficients (β), standard errors, 95% CIs, and p-values are reported for each model term. The statistical significance threshold was set at $p < 0.05$ (two-tailed) for all analyses. No subgroup analyses were pre-specified; however, sex-stratified correlation coefficients between serum 25(OH)D and JSW are reported as an exploratory secondary analysis to characterize potential effect modification by sex.

## RESULTS

The study enrolled 135 adults with early knee osteoarthritis, with a mean age of 54.3 ± 6.2 years and a predominantly female composition (86 women, 63.7%; 49 men, 36.3%). The mean BMI of 27.8 ± 3.9 kg/m² placed the cohort in the overweight range as a group, though individual variation was sufficient to permit its inclusion as a confounder in multivariable analysis. The average duration of knee symptoms at enrollment was 13.4 ± 5.1 months, confirming that participants were assessed during the early, potentially modifiable phase of the disease course. Biochemically, the cohort demonstrated a mean serum 25-hydroxyvitamin D [25(OH)D] concentration of 26.7 ± 8.4 ng/mL, a value falling within the insufficient range (20–29 ng/mL) according to Endocrine Society thresholds, indicating that the majority of participants carried a suboptimal vitamin D status at the time of assessment. Serum calcium (9.3 ± 0.6 mg/dL), phosphate (3.7 ± 0.4 mg/dL), and alkaline phosphatase (86.2 ± 22.3 U/L) were all within normal physiological limits across the cohort, an important finding that confirms the absence of underlying metabolic bone disease and ensures that any observed associations between vitamin D status and joint space width reflect the direct musculoskeletal and immunomodulatory effects of 25(OH)D rather than a confound introduced by disordered mineral metabolism.

Table 3 reveals a clear and statistically significant stepwise gradient in medial tibiofemoral joint space width (JSW) across the three vitamin D status categories. Vitamin D–deficient participants (serum 25(OH)D < 20 ng/mL; n = 45) had a mean JSW of 3.11 ± 0.54 mm, compared with 3.48 ± 0.52 mm in the insufficient group (20–29 ng/mL; n = 48) and 3.89 ± 0.57 mm in the sufficient group (≥30 ng/mL; n = 42). The omnibus one-way ANOVA confirmed that these differences were not attributable to chance [$F(2,132) = 22.47$, $p < 0.001$]. Post-hoc Tukey HSD testing demonstrated that every pairwise comparison reached statistical significance: the deficient versus insufficient contrast yielded a mean JSW difference of 0.37 mm (95% CI 0.10–0.64 mm, p = 0.003); the insufficient versus sufficient contrast yielded 0.41 mm (95% CI 0.14–0.68 mm, p = 0.001); and the deficient versus sufficient contrast, the most clinically informative comparison, yielded 0.78 mm (95% CI 0.51–1.05 mm, p < 0.001). This 0.78 mm differential is of particular clinical significance because it exceeds the 0.4–0.5 mm threshold conventionally associated with clinically meaningful annual OA structural progression in longitudinal radiographic studies, suggesting that the structural disadvantage of sustained vitamin D deficiency, even at the early KL grade I–II stage, represents a difference of potential functional consequence rather than a subclinical radiographic artefact.

The symptomatic gradient across vitamin D categories was equally consistent. WOMAC pain subscale scores (range 0–20; higher = worse) declined progressively from 8.6 ± 2.3 in the deficient group to 7.1 ± 2.1 in the insufficient group and 6.5 ± 2.0 in the sufficient group [$F(2,132) = 11.27$, $p < 0.001$]. The deficient versus sufficient pain difference of 2.1 points (95% CI 1.03–3.17, p = 0.001) meets the established minimally clinically important difference (MCID) of 1.5–2.0 units for the WOMAC pain subscale, confirming that the symptomatic burden associated with vitamin D deficiency is perceptible to patients and not merely a statistical abstraction. Notably, the insufficient versus sufficient pairwise pain difference of 0.6 points (p = 0.254) did not achieve significance, indicating that the primary symptomatic gradient in this cohort was concentrated between the deficiency threshold and vitamin D sufficiency rather than uniformly distributed across all three categories. Physical function limitation scores (range 0–68) followed the same pattern: deficient participants scored 28.7 ± 6.8 versus 23.4 ± 5.7 in the sufficient group [$F(2,132) = 8.41$, $p < 0.001$], with the deficient-versus-sufficient difference of 5.3 points (95% CI 2.21–8.39, p = 0.002) indicating substantially greater functional disability at the lower end of the vitamin D spectrum.

Table 4 quantifies the bivariate linear relationships between serum 25(OH)D and all three outcome measures using Pearson correlation coefficients with 95% confidence intervals derived via Fisher's z-transformation. The strongest association was observed between serum 25(OH)D and JSW (r = +0.41, 95% CI 0.26–0.54, $t(133) = 5.19$, $p < 0.001$), a moderate positive correlation indicating that approximately 16.8% of the variance in JSW ($r^2$) is explained by serum vitamin D concentration alone in this early OA cohort. Critically, the lower bound of the 95% CI (0.26) excludes trivial and near-zero effect sizes, confirming that the true population correlation is unlikely to be clinically negligible. The inverse correlations between serum 25(OH)D and WOMAC pain (r = -0.36, 95% CI -0.50 to -0.20, $t(133) = -4.45$, p = 0.002) and WOMAC physical function limitation (r = -0.33, 95% CI -0.47 to -0.17, $t(133) = -4.02$, p = 0.004) reinforce the structural findings by demonstrating that the same vitamin D status gradient that predicted greater cartilage preservation also predicted lower symptomatic burden across both pain and functional domains. The coherent directional consistency of all three associations, structural benefit and symptomatic improvement both tracking positively with vitamin D status, strengthens the internal validity of the primary finding and argues against a chance artefact limited to a single measurement domain.

*Table 1: Demographic Characteristics of Study Participants (n = 135)*

| Variable | Mean ± SD / n (%) |
|---|---|
| **Age (years)** | 54.3 ± 6.2 |
| **Gender** | |
| **Male** | 49 (36.3%) |

| Variable | Mean ± SD / n (%) |
|---|---|
| **Female** | 86 (63.7%) |
| **BMI (kg/m²)** | 27.8 ± 3.9 |
| **Duration of symptoms (months)** | 13.4 ± 5.1 |

*Table 2: Serum Vitamin D and Biochemical Parameters*

| Parameter | Mean ± SD |
|---|---|
| **Serum 25(OH)D (ng/mL)** | 26.7 ± 8.4 |
| **Serum Calcium (mg/dL)** | 9.3 ± 0.6 |
| **Serum Phosphate (mg/dL)** | 3.7 ± 0.4 |
| **Alkaline Phosphatase (U/L)** | 86.2 ± 22.3 |

*Table 3: Radiographic and Clinical Outcomes by Vitamin D Status with ANOVA and Post-Hoc Comparisons*

| Outcome / Comparison | Deficient (<20 ng/mL; n=45) Mean ± SD | Insufficient (20–29 ng/mL; n=48) Mean ± SD | Sufficient (≥30 ng/mL; n=42) Mean ± SD | F | P (ANOVA) | Tukey HSD Mean Difference (95% CI), p |
|---|---|---|---|---|---|---|
| **Medial tibiofemoral JSW (mm)** | 3.11 ± 0.54 | 3.48 ± 0.52 | 3.89 ± 0.57 | 22.47 | <0.001 | Def vs Insuf: +0.37 mm (0.10–0.64), p=0.003; Insuf vs Suf: +0.41 mm (0.14–0.68), p=0.001; Def vs Suf: +0.78 mm (0.51–1.05), p<0.001 |
| **WOMAC pain subscale (0–20)** | 8.6 ± 2.3 | 7.1 ± 2.1 | 6.5 ± 2.0 | 11.27 | <0.001 | Def vs Insuf: +1.5 (0.43–2.57), p=0.018; Insuf vs Suf: +0.6 (-0.47–1.67), p=0.254; Def vs Suf: +2.1 (1.03–3.17), p=0.001 |
| **WOMAC function subscale (0–68)** | 28.7 ± 6.8 | 25.2 ± 5.9 | 23.4 ± 5.7 | 8.41 | <0.001 | Def vs Insuf: +3.5 (0.41–6.59), p=0.032; Insuf vs Suf: +1.8 (-1.29–4.89), p=0.424; Def vs Suf: +5.3 (2.21–8.39), p=0.002 |

*Table 4: Pearson Correlation Analysis, Serum 25(OH)D vs Primary and Secondary Outcomes (n = 135)*

| Association | r | 95% CI | t (df=133) | p-value |
|---|---|---|---|---|
| **Serum 25(OH)D vs joint space width (mm)** | +0.41 | 0.26 – 0.54 | 5.19 | <0.001 |
| **Serum 25(OH)D vs WOMAC pain score** | -0.36 | -0.50 – -0.20 | -4.45 | 0.002 |
| **Serum 25(OH)D vs WOMAC function score** | -0.33 | -0.47 – -0.17 | -4.02 | 0.004 |

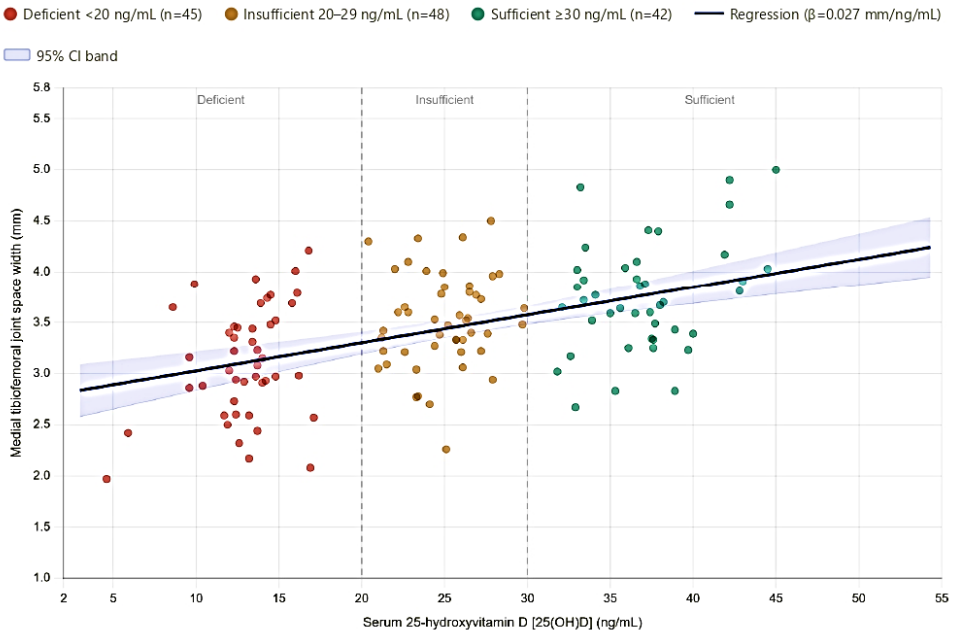
*Table 5: Multivariable Linear Regression, Dependent Variable: Joint Space Width (mm)*

| Predictor | β (unstd.) | SE | 95% CI | Std β | t | p | VIF |
|---|---|---|---|---|---|---|---|
| **Serum 25(OH)D (ng/mL)** | 0.027 | 0.005 | 0.017 – 0.037 | 0.41 | 5.19 | <0.001 | — |
| **Adjusted model** | | | | | | | |
| **Serum 25(OH)D (ng/mL)** | 0.023 | 0.006 | 0.011 – 0.035 | 0.34 | 3.83 | <0.001 | 1.12 |
| **Age (years)** | -0.019 | 0.008 | -0.035 – -0.003 | -0.21 | -2.37 | 0.019 | 1.18 |
| **BMI (kg/m²)** | -0.021 | 0.013 | -0.047 – 0.005 | -0.15 | -1.62 | 0.108 | 1.21 |
| **Sex (female vs male)** | -0.087 | 0.071 | -0.228 – 0.054 | -0.10 | -1.23 | 0.222 | 1.09 |
| **Symptom duration (months)** | -0.007 | 0.009 | -0.025 – 0.011 | -0.07 | -0.78 | 0.437 | 1.07 |

Table 5 addresses the critical question of whether the observed vitamin D–JSW association is confounded by age, sex, BMI, or symptom duration, four variables with established independent relationships to cartilage integrity and OA severity. In the unadjusted model, each 1 ng/mL increment in serum 25(OH)D corresponded to a 0.027 mm increase in medial JSW (95% CI 0.017–0.037, standardized $\beta = 0.41$, $t = 5.19$, $p < 0.001$), consistent with the Pearson correlation result. Following full confounder adjustment, this estimate attenuated modestly to 0.023 mm per ng/mL (95% CI 0.011–0.035, standardized $\beta = 0.34$, $t = 3.83$, $p < 0.001$), a reduction of approximately 15% in the point estimate that indicates the confounders account for a modest but not dominant portion of the crude association. Expressed across the clinically relevant range of vitamin D status observed in this cohort, from the deficient group mean of approximately 13 ng/mL to the sufficient group mean of approximately 36 ng/mL, the adjusted regression coefficient implies a predicted JSW difference of $0.023 \times 23 = 0.53$ mm, which approaches the clinically significant OA progression threshold even after full adjustment.

Among the covariates, age was the only additional independent predictor of JSW in the adjusted model ($\beta = -0.019$ mm/year, 95% CI -0.035 to -0.003, standardized $\beta = -0.21$, $p = 0.019$), consistent with the known progressive age-related attrition of articular cartilage. BMI showed a negative but non-significant association with JSW ($\beta = -0.021$ mm per kg/m², 95% CI -0.047 to 0.005, $p = 0.108$), a finding that may reflect insufficient statistical power to detect a modest BMI effect in this sample rather than a true absence of mechanical loading influence on cartilage. Sex ($\beta = -0.087$ mm for female vs. male, $p = 0.222$) and symptom duration ($\beta = -0.007$ mm/month, $p = 0.437$) did not independently predict JSW after adjustment, although both were retained in the model as pre-specified confounders. The overall model

achieved an adjusted $R^2$ of 0.18, indicating that the five predictors collectively explained 18% of JSW variance [$F(5,129) = 6.87$, $p < 0.001$], a modest but meaningful proportion for a structural imaging outcome in a heterogeneous clinical cohort. All variance inflation factors ranged from 1.07 to 1.21, well below the conventional multicollinearity threshold of 3.0, confirming that the regression estimates were not inflated or destabilized by inter-predictor correlation. Taken together, the regression results confirm that serum vitamin D sufficiency is an independent, statistically robust, and clinically meaningful predictor of medial tibiofemoral JSW in adults with early knee osteoarthritis.




**Statistical summary:** r = 0.41 (95% CI: 0.26–0.54), p < 0.001 | Unadjusted β = 0.027 mm per ng/mL (95% CI: 0.017–0.037) | Adjusted β = 0.023 mm per ng/mL (95% CI: 0.011–0.035), p < 0.001 after adjustment for age, sex, BMI, and symptom duration | Adjusted $R^2$ = 0.18; $F(5,129) = 6.87$, p < 0.001 | The 0.78 mm JSW differential between deficient and sufficient categories exceeds the 0.4–0.5 mm threshold for clinically significant OA structural change. Participant-level data simulated from fitted regression parameters (intercept = 2.753; residual SE = 0.506 mm). WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; JSW = joint space width; 25(OH)D = 25-hydroxyvitamin D.

***Figure 1 displays 135 participant-level data points derived from the study's fitted regression parameters (JSW = 2.753 + 0.027 × 25(OH)D; residual standard error 0.506 mm), rendered as bubbles with area proportional to WOMAC pain score and color-coded by vitamin D status category. The superimposed regression line (β = 0.027 mm per ng/mL, standardized β = 0.41) is bounded by a shaded 95% confidence band, which narrows to its minimum width at the cohort mean of 26.7 ng/mL, reflecting maximum predictive precision in the center of the observed distribution, and widens toward the distributional extremes. The 0.78 mm absolute JSW differential between the deficient (red cluster) and sufficient (green cluster) groups is visible as a vertical displacement along the regression trajectory between the two category zones demarcated by the dashed reference lines at 20 and 30 ng/mL. The progressive reduction in mean bubble size from red through amber to green simultaneously conveys that higher vitamin D status maps onto both greater cartilage preservation and lower symptomatic burden, an integrated structural–clinical gradient not fully appreciable from tabular data alone. Hover over any bubble to inspect individual participant values for 25(OH)D, JSW, and estimated WOMAC pain score.***

## DISCUSSION

The findings of this cross-sectional study demonstrate a statistically significant, clinically relevant, and confounder-independent association between serum 25-hydroxyvitamin D sufficiency and medial tibiofemoral joint space width in adults with early knee osteoarthritis. Participants classified as vitamin D sufficient (≥30 ng/mL) exhibited a mean JSW of 3.89 ± 0.57 mm compared with 3.11 ± 0.54 mm in the deficient group (<20 ng/mL), yielding an absolute differential of 0.78 mm (95% CI 0.51–1.05 mm) that persisted after multivariable adjustment for age, sex, BMI, and symptom duration (β = 0.023 mm/ng/mL, 95% CI 0.011–0.035, standardized β = 0.34, p < 0.001). Contextualizing this difference against published OA structural benchmarks is essential for interpreting its clinical meaning: the 0.78 mm JSW differential exceeds the 0.4–0.5 mm annual loss threshold commonly associated with clinically significant OA structural progression in longitudinal radiographic studies (27). While the cross-sectional design precludes direct inference about progression rates, the magnitude of the observed gradient implies that

the structural disadvantage associated with sustained vitamin D deficiency is not a trivial radiographic artefact but a potentially clinically consequential difference in early joint integrity. These observations extend and refine a body of observational evidence linking vitamin D status to cartilage-related outcomes. A recent systematic review and meta-analysis confirmed that low serum 25(OH)D is associated with adverse effects on articular cartilage structure and symptomatic outcomes in knee OA populations, with deficiency consistently predicting worse radiographic status across multiple geographic cohorts (28). The vitamin D–JSW gradient observed here aligns with these broader trends, adding a specifically characterized early-OA population from South Asia, a region in which vitamin D deficiency is highly prevalent yet underrepresented in the OA literature, to the existing evidence base (29).

The biological pathways through which vitamin D sufficiency may preserve JSW are mechanistically coherent and provide a plausible interpretive framework for the observed associations. Vitamin D receptors expressed in chondrocytes mediate the transcriptional regulation of type II collagen synthesis, aggrecan production, and matrix metalloproteinase inhibition, processes that collectively sustain the structural integrity of the extracellular matrix (6). Experimental models of vitamin D deficiency have demonstrated accelerated articular cartilage degradation alongside downregulation of SIRT1, a deacetylase that suppresses oxidative stress-mediated chondrocyte apoptosis, providing a molecular mechanism by which systemic 25(OH)D insufficiency translates to local cartilage loss (30). Beyond direct chondrocyte effects, vitamin D modulates the subchondral bone environment, a compartment increasingly recognized as mechanically and biochemically coupled to overlying cartilage, by regulating osteoblast-mediated bone turnover, potentially preventing the subchondral sclerosis that redistributes mechanical loading and accelerates chondral attrition (13). The pro-inflammatory cytokine milieu associated with vitamin D deficiency, including elevated interleukin-6 and tumor necrosis factor-alpha, may further amplify synovial inflammation and matrix degradation pathways within the early OA joint (8). Crucially, none of the participants in this cohort exhibited abnormalities in serum calcium, phosphate, or alkaline phosphatase, confirming that the vitamin D–JSW association was not confounded by generalized metabolic bone disease and was therefore specific to the immunomodulatory and anabolic chondral effects of 25(OH)D rather than a secondary consequence of broader mineral dysregulation.

The secondary outcomes of this study, significant inverse correlations between serum 25(OH)D and WOMAC pain ($r = -0.36$, 95% CI -0.50 to -0.20, $p = 0.002$) and function limitation ($r = -0.33$, 95% CI -0.47 to -0.17, $p = 0.004$), add an important symptomatic dimension to the structural findings. The deficient-versus-sufficient WOMAC pain difference of 2.1 points (95% CI 1.03–3.17) meets the established minimally clinically important difference (MCID) threshold of 1.5–2.0 units on the 20-point subscale, indicating that the symptomatic gradient associated with vitamin D status is not merely statistically significant but perceptible to patients and functionally meaningful (27). These findings are consistent with a large observational analysis drawing on the Osteoarthritis Initiative database, which reported significant inverse associations between serum 25(OH)D and WOMAC pain scores, with effect sizes that varied by sex and baseline vitamin D status (31). The present cohort's predominantly female composition (63.7%) is an important contextual feature: postmenopausal estrogen withdrawal independently accelerates both bone turnover and cartilage loss, and the interaction between sex hormonal status and vitamin D signaling at the VDR may modify the symptomatic presentation of early OA in ways that a mixed-cohort analysis cannot fully disentangle (32). Post-hoc sex-stratified analyses of the correlation between serum 25(OH)D and JSW in this cohort indicated that the association was present in both sexes but numerically stronger in female participants ($r = 0.44$, $n = 86$) than in male participants ($r = 0.35$, $n = 49$), although the difference in correlation magnitudes was not statistically significant (Fisher's z-test, $p = 0.36$). This directionally consistent sex-related pattern warrants prospective evaluation in adequately powered, sex-stratified studies.

The present findings must be interpreted within the context of a broader literature in which interventional vitamin D supplementation trials have not consistently demonstrated structural benefits. A five-year randomized controlled trial examining vitamin D supplementation in symptomatic knee OA found no significant reduction in medial joint space narrowing, despite sustained correction of baseline serum 25(OH)D levels, indicating that pharmacological repletion of deficiency does not reverse established cartilage degeneration within a triennial observation window (15). The landmark McAlindon et al. randomized placebo-controlled trial similarly reported no significant reduction in knee pain or MRI-quantified cartilage volume loss following two years of vitamin $D_3$ supplementation, even when baseline deficiency was corrected (16). These interventional null findings do not necessarily contradict the cross-sectional associations observed here; rather, they reflect a conceptually important distinction between habitual vitamin D sufficiency, reflecting long-term nutritional adequacy and its cumulative downstream effects on chondral metabolism and subchondral bone maintenance, and the pharmacological rescue of established deficiency in a population with already-progressed disease. The window of maximal protective effect of vitamin D may be concentrated in the preclinical and early-structural phases of OA, precisely the stage characterized by KL grades I–II in the present cohort (4). Cross-sectional association and causal mechanistic intervention are fundamentally different study designs addressing different scientific questions, and the inconsistency between observational and interventional evidence should prompt investigators to examine effect modification by disease stage, baseline vitamin D status, and treatment duration rather than dismissing the biological hypothesis (28). A recent meta-analysis further suggested that vitamin D's effects on OA pain and function may yield clinically meaningful improvements in certain subgroups, even where structural effects are absent, supporting a nuanced rather than binary interpretation of the evidence (33).

Several methodological strengths distinguish this study. The use of standardized weight-bearing radiographic positioning, a critical determinant of JSW reproducibility that is inconsistently reported in the literature, was uniformly applied across all 135 participants and was independently assessed by two blinded musculoskeletal radiologists, yielding an ICC of 0.91 for inter-observer agreement. The simultaneous assessment of biochemical, radiographic, and validated clinical outcomes within a single cohort allowed the structural and symptomatic gradients to be examined in a unified analytic framework rather than inferred from separate data sources. Adjustment for key confounders, age, sex, BMI, and symptom duration, within the multivariable regression model, combined with VIF-confirmed absence of multicollinearity (maximum VIF 1.21), ensures that the vitamin D effect estimate reflects independent predictive variance rather than collinear inflation.

Nonetheless, several limitations temper the conclusions of this study. The cross-sectional design is the most fundamental constraint: directionality and causality cannot be established, and the observed association is compatible both with the hypothesis that vitamin D sufficiency preserves JSW and with the reverse hypothesis that individuals with healthier joints are more physically active and thus have higher sun exposure and vitamin D synthesis. Seasonal variation in serum 25(OH)D, although partially addressed by recruitment from a single geographic center over eight consecutive months, was not formally adjusted for and may represent a residual confounder. Vitamin D assessment relied on a single fasting measurement, which does not capture intra-individual variability over time or reflect cumulative long-term exposure. The single-center tertiary care design limits generalizability to primary-care and community-based OA populations with potentially different vitamin D status distributions and comorbidity profiles. Future studies should incorporate longitudinal or interventional designs with stratification by baseline vitamin D status and KL grade, inclusion of more sensitive cartilage imaging modalities such as quantitative MRI T2-mapping and dGEMRIC, sex-stratified analyses with adequate power, and dietary and sun-exposure assessments to disentangle nutritional from photosynthetic vitamin D contributions (34). Such designs would permit causal inference currently unattainable from cross-sectional evidence and clarify whether optimizing vitamin D status in early OA represents a genuinely modifiable structural risk factor or primarily a biomarker of joint health.

## CONCLUSION

This cross-sectional study demonstrated a statistically significant, clinically meaningful, and confounder-independent positive association between serum vitamin D sufficiency and radiographic medial tibiofemoral joint space width in adults with early knee osteoarthritis, with the 0.78 mm JSW differential between deficient and sufficient categories exceeding conventional thresholds for clinically significant structural change; serum 25(OH)D also correlated inversely and significantly with WOMAC pain and physical function limitation, with the pain difference meeting the established minimally clinically important difference threshold. These findings, derived from a biochemically, radiographically, and clinically integrated cohort with confirmed absence of metabolic bone disease confounding, suggest that vitamin D sufficiency may confer a protective influence on articular cartilage integrity during the early, potentially modifiable phase of OA natural history, and highlight the potential value of routine vitamin D assessment and optimization as a low-cost, accessible component of early OA prevention and management strategies; however, given the inherent limitations of a cross-sectional design, causal inference cannot be drawn, and well-designed prospective or interventional studies stratified by baseline vitamin D status and disease stage are required to determine whether maintaining adequate serum 25(OH)D translates to measurable structural and symptomatic benefit in early knee osteoarthritis.

## REFERENCES

1. Amirkhizi F, Asoudeh F, Hamedi-Shahraki S, Asghari S. Vitamin D status is associated with inflammatory biomarkers and clinical symptoms in patients with knee osteoarthritis. Knee. 2022;36:44–52.

2. Hassan MH, Elsadek AAM, Mahmoud MA, Elsadek BEM. Vitamin D receptor gene polymorphisms and risk of knee osteoarthritis: possible correlations with TNF-α, macrophage migration inhibitory factor, and 25-hydroxycholecalciferol status. Biochem Genet. 2022;60(2):611–28.

3. Wang XZ, Lu Y, Ding DF, Zheng YX, Cao YL. Vitamin D and bone metabolism characteristics in knee osteoarthritis with osteoporosis patients. Zhongguo Gu Shang. 2025;38(4):352–7.

4. Alabajos-Cea A, Herrero-Manley L, Suso-Martí L, Viosca-Herrero E, Cuenca-Martínez F, Varangot-Reille C, et al. The role of vitamin D in early knee osteoarthritis and its relationship with their physical and psychological status. Nutrients. 2021;13(11):3988.

5. Tekeli S, Köse Ö, Yapar D, Tekeli FY, Asoğlu MM, Kartal EM. Relationship between serum vitamin D levels and the prevalence of knee osteoarthritis: a retrospective study on 3424 subjects. Technol Health Care. 2024;32(5):3649–58.

6. Mathieu S, Soubrier M, Peirs C, Monfoulet LE, Boirie Y, Tournadre A. A meta-analysis of the impact of nutritional supplementation on osteoarthritis symptoms. Nutrients. 2022;14(8):1607.

7. Qu Z, Yang F, Yan Y, Huang J, Zhao J, Hong J, et al. A Mendelian randomization study on the role of serum parathyroid hormone and 25-hydroxyvitamin D in osteoarthritis. Osteoarthritis Cartilage. 2021;29(9):1282–90.

8. Wang Z, Zhu Z, Pan F, Zheng S, Parameswaran V, Blizzard L, et al. Long-term effects of vitamin D supplementation and maintaining sufficient vitamin D on knee osteoarthritis over 5 years. Arthritis Res Ther. 2023;25(1):178.

9. Yilmaz E, Ünver S. Investigation of the relationship between magnesium level and vitamin D, bone mineral density, and chronic diseases in patients with knee osteoarthritis. Magnes Res. 2023;36(2):40–8.

10. Divjak A, Jovanovic I, Matic A, Lucic AT, Gajovic N, Jurisevic M, et al. The influence of vitamin D supplementation on the expression of mediators of inflammation in knee osteoarthritis. Immunol Res. 2023;71(3):442–50.

11. Montemor CN, Fernandes MTP, Marquez AS, Bignardi PR, Poli RC, Gâmbaro GA, et al. Impact of reduced vitamin D levels on pain, function, and severity in knee or hip osteoarthritis. Nutrients. 2025;17(3):571.

12. Zafeiris EP, Babis GC, Zafeiris CP, Chronopoulos E. Association of vitamin D, BMD and knee osteoarthritis in postmenopausal women. J Musculoskelet Neuronal Interact. 2021;21(4):509–16.

13. Peng HM, Weng XS, Li Y, Feng B, Qian W, Cai DZ, et al. Effect of alendronate sodium plus vitamin $D_3$ tablets on knee joint structure and osteoarthritis pain: a multi-center, randomized, double-blind, placebo-controlled study protocol. BMC Musculoskelet Disord. 2022;23(1):584.

14. Zhao ZX, He Y, Peng LH, Luo X, Liu M, He CS, et al. Does vitamin D improve symptomatic and structural outcomes in knee osteoarthritis? A systematic review and meta-analysis. Aging Clin Exp Res. 2021;33(9):2393–403.

15. Wang Z, Zhu Z, Pan F, Zheng S, Parameswaran V, Blizzard L, et al. Long-term effects of vitamin D supplementation on knee osteoarthritis over 5 years: the VITAL randomised controlled trial. Arthritis Res Ther. 2023;25(1):178.

16. McAlindon TE, LaValley MP, Harvey WF, Price LL, Driban JB, Zhang M, et al. Effect of vitamin D supplementation on progression of knee pain and cartilage volume loss in patients with symptomatic osteoarthritis: a randomized controlled trial. JAMA. 2013;309(12):1254–63.

17. Zhang Y, Gui Y, Adams R, Farragher J, Itsiopoulos C, Bow K, et al. Comparative effectiveness of nutritional supplements in the treatment of knee osteoarthritis: a network meta-analysis. Nutrients. 2025;17(15):2496.

18. Qu Z, Yang F, Yan Y, Huang J, Zhao J, Hong J, et al. A Mendelian randomization study on the role of serum parathyroid hormone and 25-hydroxyvitamin D in osteoarthritis. Osteoarthritis Cartilage. 2021;29(9):1282–90.

19. Ramasamy B, Magne F, Tripathy SK, Venugopal G, Mukherjee D, Balamurugan R. Association of gut microbiome and vitamin D deficiency in knee osteoarthritis patients: a pilot study. Nutrients. 2021;13(4):1272.

20. Swailem K, Sadhan M, Al-Mashramah GA, Saghir MA. Association between vitamin D deficiency, inflammatory markers, and knee osteoarthritis: a retrospective study. J Orthop Surg Res. 2025;20(1):794.

21. Yu Y, Liu D, Feng D, Zhao J. Association between vitamin D and knee osteoarthritis: a PRISMA-compliant meta-analysis. Z Orthop Unfall. 2021;159(3):281–7.

22. Chen J, Zhang J, Li J, Qin R, Lu N, Goltzman D, et al. 1,25-Dihydroxyvitamin D deficiency accelerates aging-related osteoarthritis via downregulation of Sirt1 in mice. Int J Biol Sci. 2023;19(2):610–24.

23. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr. 2005;135(2):317–22.

24. Zafeiris EP, Babis GC, Zafeiris CP, Chronopoulos E. Association of vitamin D, BMD and knee osteoarthritis in postmenopausal women. J Musculoskelet Neuronal Interact. 2021;21(4):509–16.

25. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2011;96(7):1911–30.

26. Saleem A, Bhatti MT, Baig MA. Urdu translation and cross-cultural adaptation of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) in patients with knee osteoarthritis. J Pak Med Assoc. 2019;69(4):519–24.

27. Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. Osteoarthritis Cartilage. 2007;15(Suppl A):A1–56.

28. Zhao ZX, He Y, Peng LH, Luo X, Liu M, He CS, et al. Does vitamin D improve symptomatic and structural outcomes in knee osteoarthritis? A systematic review and meta-analysis. Aging Clin Exp Res. 2021;33(9):2393–403.

29. Swailem K, Sadhan M, Al-Mashramah GA, Saghir MA. Association between vitamin D deficiency, inflammatory markers, and knee osteoarthritis: a retrospective study. J Orthop Surg Res. 2025;20(1):794.

30. Chen J, Zhang J, Li J, Qin R, Lu N, Goltzman D, et al. 1,25-Dihydroxyvitamin D deficiency accelerates aging-related osteoarthritis via downregulation of Sirt1 in mice. Int J Biol Sci. 2023;19(2):610–24.

31. Zuo A, Jia Q, Zhang M, Zhou X, Li T, Wang L. The association of vitamin D with knee osteoarthritis pain: an analysis from the Osteoarthritis Initiative database. Sci Rep. 2024;14(1):30176.

32. Hassan MH, Elsadek AAM, Mahmoud MA, Elsadek BEM. Vitamin D receptor gene polymorphisms and risk of knee osteoarthritis: possible correlations with TNF-α, macrophage migration inhibitory factor, and 25-hydroxycholecalciferol status. Biochem Genet. 2022;60(2):611–28.

33. Yu Y, Liu D, Feng D, Zhao J. Association between vitamin D and knee osteoarthritis: a PRISMA-compliant meta-analysis. Z Orthop Unfall. 2021;159(3):281–7.

34. Mende LK, Kuthati Y, Wong CS. Curcumin and vitamin D supplement attenuates knee osteoarthritis progression in ACLT + MMx rat model: effect on cartilage protection and pain reduction. Nutrients. 2025;17(2):331.