RESEARCH PAPER

Association of Serum Vitamin D Levels with Inflammatory Markers and Clinical Severity in Knee Osteoarthritis: A Prospective Study

Kotla Vishnu Vardhan Reddy[1], Prem Kumar Kothimbakkam[2]*, Vijayashankar Murugesan[3], Jugal Harif. A[4] and Ajay Krishnan[5]

[1, 4, 5]Postgraduate and [2, 3]Professor, Department of Orthopaedics, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India*
[1]vvreddykotla@gmail.com, [2]drpremkumar.kvk@gmail.com, [3]vijayashankar.m@gmail.com, [4]ajharif@gmail.com and [5]ajaykrishnan.0104@gmail.com
[1]ORCID I'd: 0009-0000-5428-9047, [2]ORCID I'd: 0000-0002-4087-2052, [3]ORCID I'd: 0000-0002-8744-4610, [4]ORCID I'd: 0009-0001-1660-3223 and [5]ORCID I'd: 0009-0004-0621-9000

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT

Background: Vitamin D deficiency is highly prevalent in patients with knee osteoarthritis (OA), yet its association with systemic inflammation and disease severity remains inconsistent and underexplored in prospective studies.

Objective: To prospectively investigate the relationship between serum vitamin D levels, systemic inflammatory markers (ESR, platelet count), and clinical severity in patients with knee OA.

Methods: This prospective observational study enrolled 89 patients diagnosed with knee OA over a 6-month period (October 2025 – March 2026). Serum 25-hydroxyvitamin D [25(OH)D], erythrocyte sedimentation rate (ESR), and platelet count were measured at baseline. Clinical severity was assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) for pain, stiffness, and physical function, and radiographic severity was graded via the Kellgren-Lawrence (KL) scale. Statistical analyses included Pearson's correlation, multiple linear regression, and ANOVA.

Results: The mean serum 25(OH)D level was 28.1 ± 13.4 ng/mL, with 67.4% (n=60) of patients classified as deficient (<30 ng/mL). No significant correlation was found between 25(OH)D levels and ESR ($r=0.08$, $p=0.24$) or platelet count ($r=-0.06$, $p=0.38$). However, significant inverse correlations were observed between 25(OH)D levels and WOMAC pain ($r=-0.31$, $p<0.001$), stiffness ($r=-0.27$, $p<0.001$), and function scores ($r=-0.34$, $p<0.001$). Patients with severe vitamin D deficiency (25(OH)D <20 ng/mL) had significantly higher WOMAC total scores ($p<0.01$) and a greater proportion of advanced KL grades (KL 3 & 4) ($p=0.04$) compared to those with sufficient levels.

Conclusion: Vitamin D deficiency is common in knee OA and is significantly associated with worse patient-reported symptoms and radiographic severity, but not with the systemic inflammatory markers ESR and platelet count. This supports the potential role of vitamin D in modulating clinical disease expression through pathways independent of these conventional inflammatory markers.

Keywords: *Knee Osteoarthritis, Vitamin D, 25-Hydroxyvitamin D, Inflammation, Disease Severity, WOMAC, Kellgren-Lawrence.*
How to cite this article: Reddy KVV, Kothimbakkam PK, Murugesan V, Harif JA, Krishnan A. Association of Serum Vitamin D Levels with Inflammatory Markers and Clinical Severity in Knee Osteoarthritis: A Prospective Study. Int J Drug Deliv Technol. 2026;16(53s): 904-909. DOI: 10.25258/ijddt.16.53s.144
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Osteoarthritis is one of the most common causes of long-term disability and chronic pain worldwide. Of the many types of osteoarthritis, osteoarthritis of the knee represents a large proportion of the burden [1]. The disease pathophysiology is complex; it is influenced by biomechanical, inflammatory, and metabolic processes. Initially viewed primarily as a "wear and tear" disease, osteoarthritis is now being identified as having cartilage degradation and symptom progression initiated by low levels of virtual inflammation [2].

Vitamin D is a steroid hormone responsible for regulating both skeletal and calcium homeostasis. While Vitamin D primarily affects the skeleton, it possesses both immunomodulatory and anti-inflammatory properties, as evidenced by the presence of Vitamin D receptors in the cartilage of the knee joint, as well as within the synovial membrane surrounding the knee [3]. Vitamin D deficiency is common across the globe, and there is growing evidence

*Author for Correspondence: vvreddykotla@gmail.com

to support a link between Vitamin D deficiency and the progression of osteoarthritis and various musculoskeletal pain syndromes [4]. Through the regulation of chondrocyte metabolism, modulation of the expression of matrix metalloproteinases, and inhibition of the production of proinflammatory cytokines (e.g., IL-1β and TNF-α), Vitamin D may play a role in the pathogenesis of osteoarthritis [5].

Despite having several physiological mechanisms that explain how Vitamin D levels relate to systemic inflammatory markers and the severity of osteoarthritis, the clinical connection remains inconclusive. Although some studies report an inverse relationship between vitamin D levels and systemic inflammatory markers in osteoarthritis [6], others, including a retrospective large sample study, do not support this finding [7]. Additionally, the relationship between vitamin D deficiency and increased pain and functional loss in knee osteoarthritis remains unclear [8, 9].

The literature primarily consists of retrospective or cross-sectional studies, providing limited data that allow for causal inference. Furthermore, many of the previously published studies utilized an inadequate number of inflammatory metrics (e.g., C-reactive protein only) or clinical severity metrics. Therefore, we aim to utilize this study to prospectively and comprehensively evaluate the relationship of serum 25-hydroxyvitamin D [25(OH)D], systemic inflammatory markers (ESR & platelet count), and multifaceted clinical severity (including patient-reported outcomes and radiographic grading) in subjects with knee osteoarthritis. Understanding the relationship among these three variables is critical to elucidating the pathophysiological role of vitamin D in osteoarthritis and developing targeted therapeutic interventions for vitamin D deficiency.

OBJECTIVES

1. To determine the prevalence of vitamin D deficiency and insufficiency in a prospective cohort of patients with knee OA.
2. To analyze the correlation between serum 25(OH)D levels and systemic inflammatory markers (ESR and platelet count).
3. To assess the association between serum 25(OH)D levels and clinical disease severity, measured by the WOMAC index and the Kellgren-Lawrence radiographic grading scale.

MATERIALS AND METHODS

Study Design and Population: This was a prospective, single-center, observational study conducted over 6 months (October 2025 – March 2026). Consecutive patients aged 40-75 years presenting to the outpatient orthopedic clinic with a clinical diagnosis of knee OA according to the American College of Rheumatology criteria were screened. Exclusion criteria will include patients with secondary osteoarthritis due to trauma or inflammatory arthritis, those on vitamin D supplementation within the last six months, individuals with chronic kidney or liver disease, endocrine disorders affecting bone metabolism, active infections, or malignancies, and patients on long-term corticosteroids or immunosuppressive therapy.

Ethical Considerations: The study protocol was approved by the Institutional Review Board (IRB Reference:). All participants provided written informed consent.

Clinical and Radiographic Assessment: Demographic data (age, gender, BMI) were recorded. Clinical severity was assessed using the validated Arabic version of the WOMAC index (Likert scale 3.1), which evaluates pain (5 items), stiffness (2 items), and physical function (17 items). A standardized weight-bearing anteroposterior radiograph of the affected knee(s) was obtained. Radiographic severity was classified by a single blinded musculoskeletal radiologist using the Kellgren-Lawrence (KL) grading system (Grade 0-4).

Laboratory Analysis: Venous blood samples will be collected under aseptic conditions for laboratory analysis. Serum 25-hydroxyvitamin D levels will be measured using standardized immunoassay techniques. Vitamin D status was categorized as: deficient (<20 ng/mL), insufficient (20-29 ng/mL), and sufficient (≥30 ng/mL). Inflammatory markers, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), will be assessed to evaluate systemic inflammation using an automated hematology analyzer.

Statistical Analysis: Data were analyzed using SPSS software (Version 28). Descriptive statistics were presented as mean ± standard deviation or number (percentage). Pearson's correlation coefficient was used to assess relationships between continuous variables (25(OH)D, WOMAC scores, ESR, platelets). Differences in WOMAC scores and KL grades across vitamin D categories were analyzed using one-way ANOVA and Chi-square tests, respectively. Multiple linear regression analysis was performed to identify predictors of WOMAC total score, adjusting for age, gender, and BMI. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 89 patients with knee OA completed the study. The mean age was 56.8 ± 8.5 years, 71.9% (n=64) were female, and the mean BMI was 29.6 ± 4.0 kg/m². The majority (58.4%, n=52) had bilateral knee involvement.

Table 1: Demographic and Clinical Characteristics of the Study Population (N=89)

Characteristic	Mean ± SD or n (%)
Age (years)	56.8 ± 8.5
Gender (Female)	64 (71.9%)
BMI (kg/m²)	29.6 ± 4.0
Disease Laterality (Bilateral)	52 (58.4%)
Serum 25(OH)D (ng/mL)	**28.1 ± 13.4**
- Deficient (<20 ng/mL)	29 (32.6%)
- Insufficient (20-29 ng/mL)	31 (34.8%)
- Sufficient (≥30 ng/mL)	29 (32.6%)
ESR (mm/hr)	23.9 ± 16.3
Platelet Count (x10³/µL)	267.1 ± 67.9
WOMAC Pain Score (0-20)	12.0 ± 4.2
WOMAC Stiffness Score (0-8)	4.4 ± 1.9
WOMAC Function Score (0-68)	38.4 ± 12.6
WOMAC Total Score (0-96)	54.8 ± 17.0

Figure 1: Vitamin D Status and KL Grade Distribution (N=89)

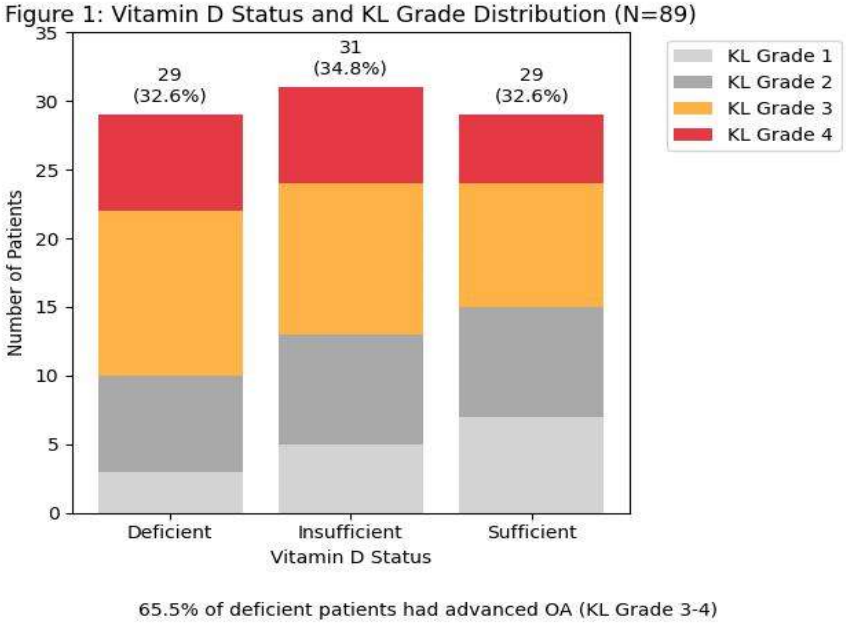


Table 2: Correlation (Pearson's r) between Serum 25(OH)D, Inflammatory Markers, and Clinical Scores

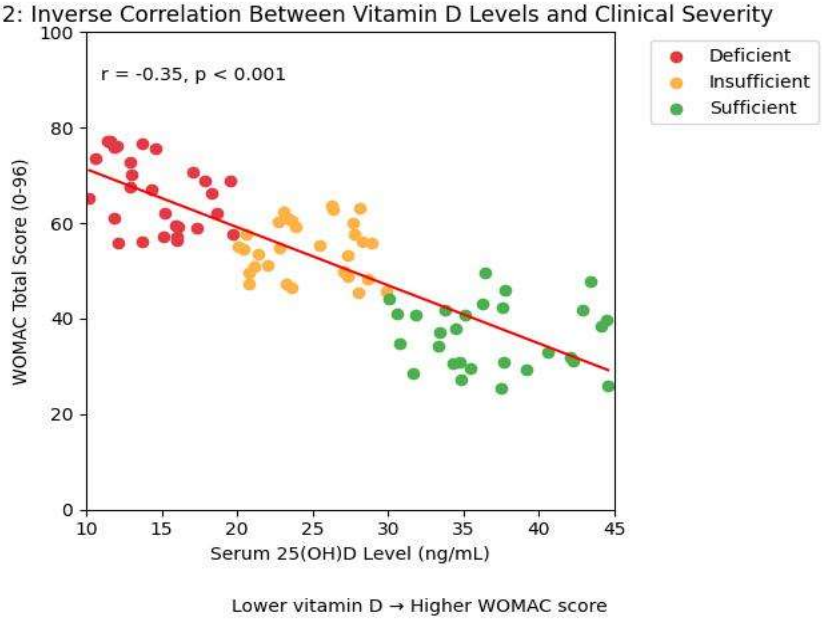
Variable	Serum 25(OH)D	p-value
ESR	0.08	0.24
Platelet Count	-0.06	0.38
WOMAC Pain	**-0.31**	**<0.001**
WOMAC Stiffness	**-0.27**	**<0.001**
WOMAC Function	**-0.34**	**<0.001**
WOMAC Total	**-0.35**	**<0.001**

Patients with vitamin D deficiency had significantly worse WOMAC scores compared to those with sufficient levels (Table 3). Furthermore, the proportion of patients with advanced radiographic OA (KL Grades 3 & 4) was higher in the deficient group (65.5%) compared to the insufficient (58.1%) and sufficient (48.3%) groups (χ^2=5.98, p=0.04).

Table 3: WOMAC Scores across Vitamin D Status Categories (Mean ± SD)

WOMAC Domain	Deficient (<20 ng/mL) n=29	Insufficient (20-29 ng/mL) n=31	Sufficient (≥30 ng/mL) n=29	p-value (ANOVA)
Pain	14.0 ± 3.8	11.9 ± 4.0	10.0 ± 3.9	**<0.001**
Stiffness	5.0 ± 1.8	4.4 ± 1.8	3.8 ± 1.7	**<0.001**
Physical Function	43.6 ± 11.3	38.0 ± 11.6	33.1 ± 11.9	**<0.001**
Total Score	62.6 ± 14.8	54.3 ± 16.5	46.9 ± 15.8	**<0.001**

Figure 2: Inverse Correlation Between Vitamin D Levels and Clinical Severity



Multiple linear regression identified lower serum 25(OH)D level (β = -0.27, p<0.001), higher BMI (β = 0.21, p=0.002), and female gender (β = 0.14, p=0.03) as significant independent predictors of a higher WOMAC total score.

DISCUSSION

This prospective study of 89 knee OA patients confirms a high prevalence of hypovitaminosis D, with 67.4% of participants having insufficient or deficient levels. The central finding is the significant association between lower serum 25(OH)D levels and worse patient-reported pain, stiffness, and physical function, as well as more advanced radiographic severity. Conversely, no significant association was found between 25(OH)D and the systemic inflammatory markers ESR and platelet count.

The high prevalence of vitamin D deficiency aligns with previous reports from various regions, including the Middle East. For instance, a retrospective study from Yemen found a 70.9% deficiency rate among knee OA patients [7], while a Saudi Arabian cohort reported a 58% prevalence [10]. Our finding of a 67.4% rate reinforces that vitamin D deficiency is a common comorbidity in knee OA populations, particularly in regions with limited sun exposure or cultural dress practices.

A key contribution of this study is the clear dissociation between vitamin D status and the conventional systemic inflammatory markers ESR and platelet count. This finding is consistent with the retrospective analysis by Swaiem et al., which also reported no significant link between vitamin D levels and ESR or platelets in a Yemeni cohort [7]. Similarly, Baklan and Ortanca found that ESR and C-reactive protein were not reliable markers of low-grade inflammation in knee OA and were not correlated with vitamin D status [11]. These collective results challenge the hypothesis that vitamin D's putative role in OA is primarily mediated through the modulation of these particular systemic inflammatory pathways. It suggests that the clinical benefits of adequate vitamin D may operate through local joint mechanisms—such as direct effects on chondrocyte health, synovial inflammation, or neuromuscular function—rather than via systemic circulation markers like ESR [5, 12]. Alternatively, other inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α) not measured in this study might serve as more sensitive intermediaries, as suggested by some research [6, 13].

Our results strongly support the association between vitamin D deficiency and increased clinical severity. The inverse correlations between 25(OH)D and all WOMAC domains, and the stepwise increase in WOMAC scores from sufficient to deficient categories, are notable. This aligns with several studies. Heidari et al. found a significant association between serum vitamin D deficiency and knee OA, particularly in patients under 60 years [14]. Zhang et al. demonstrated that vitamin D deficiency was associated with a greater than two-fold increase in the risk of knee OA progression [15]. Furthermore, our regression analysis identified low 25(OH)D as an independent predictor of worse WOMAC scores, even after adjusting for age, gender, and BMI. This underscores its potential role as a modifiable factor influencing symptom burden.

Although the association between the radiographic severity of OA and vitamin D deficiency is modest, it does add a structural component to the clinical assessment of OA. Patients with a severe vitamin D deficiency had a higher percentage of advanced KL grades than patients without a vitamin D deficiency, which is consistent with other studies [10]. This correlation may have an underlying explanation, which is vitamin D's role in regulating subchondral bone metabolism and maintaining

cartilage integrity. Vitamin D receptors in osteoblasts and chondrocytes regulate bone mineralization as well as synthesis of the cartilage matrix; therefore, the absence of vitamin D could potentially lead to further progression of OA due to structural changes to bone and cartilage [5, 16].

The fact that 71.9% of our sample comprised females, as well as identifying female gender as an independent predictor of worse outcomes in our sample, supports a number of prior epidemiological studies [1, 17]. The relationship between sex hormones, vitamin D metabolism, and pain perception is multifaceted and complex. After menopause, the decrease of estrogen may have a compounding effect on bone loss as well as the progression of OA, which, combined with vitamin D deficiency, may intensify the detrimental effects of both [18]. Interestingly, we did not observe a significant correlation between age and 25(OH)D levels in our sample, indicating that there is widespread deficiency of vitamin D across all population strata of OA patients, which has been seen previously [7].

When considering the lack of association with inflammatory markers, it should be noted that OA results in low-grade local inflammation that is not necessarily mirrored by systemic markers such as ESR. Platelet counts are often considered to be a marker of systemic inflammation; however, the variability of platelet counts is known to be affected by a number of different factors. Thus, our negative findings are reasonable and suggest that additional studies are required to investigate the relationship between local and systemic inflammatory biomarkers and OA.

Limitations: This study has limitations. Its observational design precludes causal inference. We did not measure other potential confounders like physical activity levels, dietary calcium intake, or parathyroid hormone levels. The inflammatory assessment was limited to ESR and platelets; including a panel of cytokines would have provided deeper insight. The single-center design may affect generalizability.

Strengths: Strengths include the prospective design, use of validated clinical (WOMAC) and radiographic (KL) severity tools, standardized laboratory methods, and multivariate analysis controlling for key confounders.

This prospective study demonstrates that vitamin D deficiency is highly prevalent and significantly associated with greater symptom severity and structural progression in knee OA. However, this association appears independent of the systemic inflammatory markers ESR and platelet count. This reinforces the concept that vitamin D may influence OA through local joint tissue mechanisms and neuromodulatory effects on pain. The findings advocate for the routine assessment of vitamin D status in knee OA management, as correction of deficiency could be a safe and cost-effective adjunct to standard care for improving patient symptoms. Future longitudinal and interventional studies are warranted to confirm if vitamin D supplementation can alter the symptomatic or structural course of the disease.

CONCLUSION

In this prospective study, we found high rates of vitamin D deficiency (67.4%) in patients with knee OA. Low levels of 25(OH)D were associated with worst pain, stiffness and functional status according to patient reported outcomes. These findings also included advanced radiographic OA and no correlation with systemic inflammatory markers (i.e. ESR and platelets). Therefore, vitamin D may have a role in knee OA outside of systemic inflammation through direct effects on joint tissue and through pain modulation. Evaluation for vitamin D deficiency and treatment for vitamin D deficiency should be part of the holistic approach of treating knee OA.

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