Original Article

Vitamin D Status and Its Association with Inflammatory Markers: A Biochemical Perspective

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Abstract

Background: Vitamin D is a fat-soluble secosteroid hormone with established roles in calcium–phosphate metabolism and emerging roles in immune regulation and inflammation. Low vitamin D levels have been linked to chronic inflammatory states, metabolic disorders, and autoimmune diseases. The objective is to assess vitamin D status and evaluate its association with selected inflammatory markers among 100 cases from a biochemical perspective. **Material and Methods:** A cross-sectional analytical study was conducted on 100 subjects attending a tertiary care hospital. Serum 25-hydroxyvitamin D [25(OH)D] levels and inflammatory markers including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and interleukin-6 (IL-6) were measured. Subjects were categorized based on vitamin D status as deficient, insufficient, or sufficient. Statistical analysis was performed to determine correlations between vitamin D and inflammatory markers. **Results:** Among 100 cases, vitamin D deficiency was observed in 58%, insufficiency in 26%, and sufficiency in 16%. Mean CRP, ESR, and the group that didn't get enough vitamin D had far greater levels of IL-6 than the group that did. There was a strong negative relationship between serum vitamin D levels and CRP ($r = -0.48$), ESR ($r = -0.42$), and IL-6 ($r = -0.45$). **Conclusion:** Vitamin D deficiency is highly prevalent and is significantly associated with elevated inflammatory markers. These findings suggest a potential role of vitamin D in modulating systemic inflammation and highlight the need for routine screening and correction of vitamin D deficiency.

Keywords: Vitamin D, Inflammation, CRP, ESR, IL-6, Biochemical markers.

Received: 09 March 2026 **Revised: 28 March 2026** **Accepted: 13 April 2026** **Published: 23 April 2026**

Introduction

Vitamin D is a fat-soluble secosteroid hormone that is well known for its important function in keeping calcium and phosphorus levels in balance and keeping bones healthy. However, in recent decades, growing biochemical and clinical evidence has highlighted its broader physiological significance, particularly in immune regulation, inflammatory pathways, and chronic disease modulation. Vitamin D exists in two major forms—vitamin D_2 (ergocalciferol) obtained from plant sources and vitamin D_3 (cholecalciferol) synthesized in the skin upon exposure to ultraviolet-B radiation. These inert precursors are hydroxylated in the liver and kidneys in a series of steps to make 25-hydroxyvitamin D [25(OH)D], which is the main form that circulates in the body, and then 1,25-dihydroxyvitamin D (calcitriol), which is the metabolite that is biologically active.[1]

Because it has a long half-life and consistent circulating concentration, serum 25(OH)D is commonly considered to be the best biochemical marker of vitamin D status. Beyond its classical endocrine actions, vitamin D exerts pleiotropic effects on multiple organ systems through the presence of vitamin D receptors (VDRs) in a wide variety of tissues, including immune cells such as macrophages, dendritic cells, B lymphocytes, and T lymphocytes. These receptors enable vitamin D to modulate both innate and adaptive immune responses. Calcitriol influences gene expression involved in cytokine production, immune cell proliferation, and inflammatory signaling pathways.[2]

Inflammation is a complex biological response triggered by infection, tissue injury, or metabolic disturbances. It involves activation of immune cells and release of inflammatory mediators such as cytokines and acute-phase proteins.[3] Chronic low-grade inflammation has been increasingly recognized as a central mechanism underlying the pathogenesis of several non-communicable diseases, including diabetes mellitus, cardiovascular disease, chronic kidney disease, metabolic syndrome, autoimmune disorders, and certain malignancies. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and interleukin-6 (IL-6) are some of the most prevalent biochemical markers of systemic inflammation. Elevated levels of these markers reflect ongoing inflammatory activity and have

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DOI:
10.21276/amit.2026.v13.i1.606

How to cite this article: Waghmode A, Gaikwad P, Bobade VR. Vitamin D Status and Its Association with Inflammatory Markers: A Biochemical Perspective. Acta Med Int. 2026;13(1):1105-1109.

been associated with increased disease risk and poor clinical outcomes.[4]

Recent research indicates that vitamin D functions as an immunomodulator by inhibiting pro-inflammatory cytokines, including interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and interferon-gamma, while enhancing anti-inflammatory cytokines. Vitamin D deficiency has been linked to increased inflammatory activity, impaired immune regulation, and susceptibility to chronic inflammatory conditions.[5] Experimental evidence indicates that vitamin D inhibits nuclear factor-kappa B (NF-κB) activation, reduces oxidative stress, and modulates macrophage and T-cell responses, thereby exerting anti-inflammatory effects at the cellular level.[6]

Despite abundant sunlight in many tropical regions, vitamin D deficiency remains highly prevalent worldwide, including in countries with high solar exposure. Urbanization, indoor lifestyle, limited sun exposure, skin pigmentation, dietary insufficiency, and comorbid conditions contribute to this paradoxical deficiency. In India and other developing nations, studies have reported a high prevalence of hypovitaminosis D across different age groups and clinical populations. This widespread deficiency raises concerns regarding its potential role in increasing inflammatory burden and contributing to chronic disease progression.[7]

From a biological standpoint, the interplay between vitamin D levels and inflammatory indicators is of significant interest. Understanding this relationship may provide insight into the role of vitamin D as a modifiable factor influencing systemic inflammation. Early identification of vitamin D deficiency and its association with inflammatory markers could have important implications for disease prevention, clinical management, and public health strategies.[8]

Given the increasing recognition of vitamin D as an immunomodulatory hormone and the growing burden of inflammatory disorders, it becomes essential to evaluate vitamin D status in relation to biochemical indicators of inflammation.[9]

The present study aims to assess serum vitamin D levels and investigate their association with selected inflammatory markers among 100 cases in a tertiary care setting. By exploring this relationship, the study aims to add to our knowledge of vitamin D's involvement in inflammation and show how important it is for doctors to regularly check patients' biochemistry.

Objectives

1. To estimate serum vitamin D levels among the study population (100 cases).
2. To assess levels of inflammatory markers (CRP, ESR, IL-6).
3. To evaluate the association between vitamin D status and inflammatory markers.
4. To find out if there is a link between vitamin D levels in the blood and signs of inflammation.

Materials and Methods

Study Design: Cross-sectional observational study.

Study Setting: Department of Biochemistry in collaboration with Medicine OPD/IPD at a tertiary care hospital.

Sample Size: 100 cases.

Inclusion Criteria

- Adults aged 18–65 years
- Patients willing to participate
- Patients undergoing routine biochemical evaluation

Exclusion Criteria

- Patients on vitamin D supplementation
- Chronic liver disease
- Malignancy
- Acute infection
- Steroid therapy
- Pregnancy

Methods of Data Collection: Data for the present study were collected from 100 cases attending the outpatient and inpatient departments of a tertiary care hospital after obtaining informed consent. A structured proforma was used to record demographic details including age and gender, along with relevant clinical history such as comorbid conditions, medication use, and history of vitamin D supplementation. Subjects meeting the inclusion criteria were enrolled consecutively until the required sample size was achieved. Venous blood samples (approximately 5 mL) were collected under aseptic precautions after an overnight fasting period of 8–10 hours. The samples were divided appropriately for biochemical analysis. Serum was separated by centrifugation and used for estimation of 25-hydroxyvitamin D [25(OH)D] levels and inflammatory markers. Serum vitamin D levels were measured using chemiluminescence immunoassay (CLIA) method. We used the immunoturbidimetric method to find out the levels of C-reactive protein (CRP), the Westergren method to find out the levels of erythrocyte sedimentation rate (ESR), and the enzyme-linked immunosorbent assay (ELISA) to find out the levels of interleukin-6 (IL-6). All analyses were performed according to standard laboratory protocols with appropriate quality control measures. The obtained values were recorded systematically in a master data sheet for subsequent statistical analysis.

Biochemical Parameters

Serum 25(OH) Vitamin D

Vitamin D Classification

- Deficient: <20 ng/mL
- Insufficient: 20–29 ng/mL
- Sufficient: ≥30 ng/mL

C-reactive protein (CRP) –Normal: < 5 mg/L

ESR –Normal range adult males: 0–15 mm/hour

Normal range adult females: 0–20 mm/hour

Interleukin-6 (IL-6) –Normal: < 7 pg/mL

Statistical Analysis: We put the data we collected into Microsoft Excel and used Statistical Package for the Social Sciences (SPSS) version 25.0 to look at it. We used mean ± standard deviation (SD) to show continuous variables like serum vitamin D, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and interleukin-6 (IL-6). We used frequencies and percentages to show categorical variables like gender and vitamin D status categories (deficient, insufficient, sufficient). The study population was divided into groups based on vitamin D status using standard cut-off values. The normality of distribution of continuous variables was assessed using the Shapiro–Wilk test. For comparison of mean inflammatory

marker levels among different vitamin D groups, one-way analysis of variance (ANOVA) was applied for normally distributed data, followed by post hoc Tukey's test where appropriate. In cases where data were not normally distributed, the Kruskal–Walli's test was used. We used Pearson's correlation coefficient for parametric data and Spearman's rank correlation for non-parametric data to look at the link between serum vitamin D levels and inflammatory markers. A p-value below 0.05 was deemed statistically significant. Graphical representations such as bar diagrams and scatter plots were used to illustrate the relationship between vitamin D levels and inflammatory markers.

Results

Table 1: Demographic Characteristics of Study Population (n = 100)

Variable	Number (n)	Percentage (%)
Age Group (years)		
18–30	22	22%
31–45	34	34%
46–60	28	28%
>60	16	16%
Gender		
Male	54	54%
Female	46	46%

Of the 100 instances, most (34%) were between the ages of 31 and 45, and 28% were between the ages of 46 and 60. Males constituted 54% of the study population, while females accounted for 46%.

Table 2: Distribution of Vitamin D Status (n = 100)

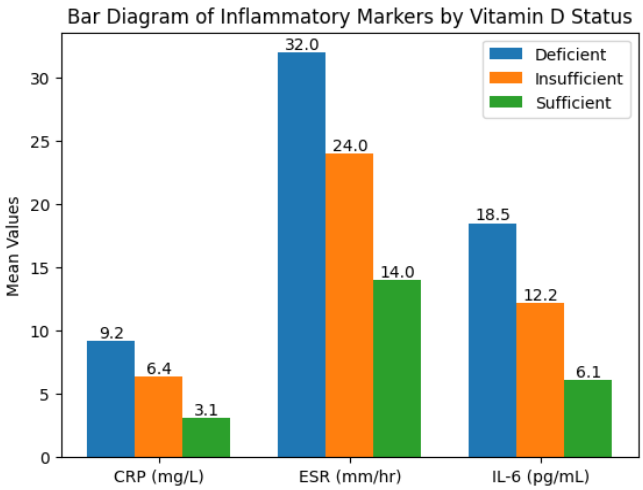
Vitamin D Category	Serum 25(OH)D Level (ng/mL)	Number (n)	Percentage (%)
Deficient	<20	58	58%
Insufficient	20–29	26	26%
Sufficient	≥30	16	16%

The distribution of vitamin D status among the 100 study participants revealed a high prevalence of suboptimal vitamin D levels. A total of 58 cases (58%) were found to be vitamin D deficient, with serum 25-hydroxyvitamin D [25(OH)D] levels below 20 ng/mL. An additional 26 cases (26%) had insufficient vitamin D levels, ranging between 20–29 ng/mL. Only 16 cases (16%) demonstrated sufficient vitamin D levels (≥30 ng/mL).

Overall, 84% of the study population had either deficient or insufficient vitamin D levels, indicating a widespread prevalence of hypovitaminosis D in the studied group. This finding underscores the significant burden of vitamin D inadequacy and highlights the need for routine screening and appropriate intervention, particularly in populations at risk for inflammatory and metabolic disorders.

Table 3: Comparison of Mean Inflammatory Marker Levels According to Vitamin D Status

Parameter	Deficient (n=58)	Insufficient (n=26)	Sufficient (n=16)	p-value
CRP (mg/L)	9.2 ± 3.1	6.4 ± 2.5	3.1 ± 1.4	<0.001
ESR (mm/hr)	32 ± 10	24 ± 8	14 ± 6	<0.001
IL-6 (pg/mL)	18.5 ± 6.2	12.2 ± 4.3	6.1 ± 2.1	<0.001



The comparison of inflammatory markers across different vitamin D categories demonstrated a clear and statistically significant trend. Individuals with vitamin D deficiency (n = 58) exhibited the highest mean levels of inflammatory markers. The mean CRP level in the deficient group was 9.2 ± 3.1 mg/L, which was considerably higher compared to the insufficient group (6.4 ± 2.5 mg/L) and the sufficient group (3.1 ± 1.4 mg/L).

Similarly, the mean ESR was highest in the deficient group (32 ± 10 mm/hr), followed by the insufficient group (24 ± 8 mm/hr), and lowest in the sufficient group (14 ± 6 mm/hr).

A comparable pattern was observed for IL-6 levels, where the deficient group showed markedly elevated levels (18.5 ± 6.2 pg/mL) compared to the insufficient group (12.2 ± 4.3 pg/mL) and the sufficient group (6.1 ± 2.1 pg/mL).

The differences in CRP, ESR, and IL-6 levels among the

three vitamin D categories were statistically highly significant ($p < 0.001$). These results show that lower vitamin D levels are linked to higher levels of systemic inflammation. This suggests that there is a strong negative association between vitamin D levels and inflammatory activity.

Table 4: Correlation Between Serum Vitamin D and Inflammatory Markers (n = 100)

Parameter	Correlation Coefficient (r)	p-value
Vitamin D vs CRP	−0.48	<0.001
Vitamin D vs ESR	−0.42	<0.01
Vitamin D vs IL-6	−0.45	<0.001

Correlation analysis indicated a statistically significant unfavourable association between serum vitamin D concentrations and inflammatory markers. A moderate negative connection was noted between vitamin D and CRP ($r = −0.48$, $p < 0.001$), signifying that diminished vitamin D levels corresponded with elevated CRP concentrations.

Similarly, vitamin D showed a significant negative correlation with ESR ($r = −0.42$, $p < 0.01$), suggesting that individuals with reduced vitamin D levels tended to have elevated erythrocyte sedimentation rates.

A comparable moderate inverse correlation was also noted between vitamin D and IL-6 levels ($r = −0.45$, $p < 0.001$).

These findings demonstrate that as serum vitamin D levels decrease, inflammatory markers significantly increase. The statistically significant p-values confirm that the observed associations are unlikely to be due to chance, supporting the hypothesis that vitamin D plays a modulatory role in systemic inflammation.

Discussion

The present study included 100 cases, with the majority of participants belonging to the 31–45-year age group (34%), followed by 46–60 years (28%). This predominance of middle-aged adults is comparable with previous studies evaluating vitamin D status and inflammatory markers, which have reported higher prevalence of hypovitaminosis D and low-grade systemic inflammation in individuals between 30 and 60 years of age. Studies by Holick et al. and Forrest and Stuhldreher have shown that vitamin D deficiency is widely prevalent among adults in the middle-age group due to lifestyle factors, reduced sun exposure, and dietary insufficiency. Similarly, hospital-based biochemical studies conducted by Jorde et al. and Lu et al. reported that participants in the fourth and fifth decades constituted the major proportion of subjects assessed for vitamin D and inflammatory markers, supporting the age distribution observed in the present study. [Table 1]

In terms of gender distribution, males constituted 54% of the study population while females accounted for 46%, showing a slight male predominance. Comparable findings have been reported in several hospital-based cross-sectional studies assessing vitamin D and inflammatory status, where male participants slightly outnumbered females. However, some studies have reported a higher prevalence of vitamin D deficiency among females, attributed to reduced sunlight exposure, sociocultural clothing practices, and hormonal influences. For instance, studies by Mithal et al,[10] in the Indian population and by van Schoor and Lips et al,[11] have highlighted a high prevalence of vitamin D deficiency in both sexes, with certain populations showing female preponderance. [Table 1]

Moreover, prior studies have consistently shown a negative correlation between serum vitamin D concentrations and inflammatory markers, including C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α). Research conducted by Amer and Qayyum et al. and by Peterson and Heffernan has indicated that diminished serum vitamin D levels correlate with elevated amounts of inflammatory markers, implying a potential immunomodulatory and anti-inflammatory function of vitamin D. These results support the biochemical point of view of this investigation, which tries to find out how vitamin D levels affect inflammatory markers. Thus, the demographic characteristics and biochemical associations observed in the present study are broadly consistent with previously published literature.

The correlation analysis in this study found a statistically significant negative association between serum vitamin D levels and inflammatory indicators such CRP ($r = −0.48$, $p < 0.001$), ESR ($r = −0.42$, $p < 0.01$), and IL-6 ($r = −0.45$, $p < 0.001$). These findings are consistent with previously published literature supporting the anti-inflammatory and immunomodulatory role of vitamin D.

Amer and Qayyum have reported a similar inverse correlation between serum 25-hydroxyvitamin D and CRP.[12] who demonstrated that lower vitamin D levels were independently associated with higher CRP concentrations in asymptomatic adults. Likewise, Peterson and Heffernan,[13] found a strong negative relationship between serum vitamin D and pro-inflammatory cytokines, such as TNF-α, which supports the idea that vitamin D has anti-inflammatory properties[2].

With respect to IL-6, Ngo et al,[14] reported that vitamin D supplementation was associated with modulation of inflammatory cytokines, including IL-6, particularly in individuals with baseline deficiency. In addition, studies evaluating metabolic and cardiovascular risk populations have consistently demonstrated that hypovitaminosis D is associated with elevated IL-6 and CRP levels, indicating chronic low-grade inflammation.

Although fewer studies have specifically evaluated the direct correlation between vitamin D and ESR, elevated ESR has been reported in populations with inflammatory conditions and concurrent vitamin D deficiency, suggesting a similar inverse trend.[15] The moderate negative correlations observed in the present study (r values ranging from −0.42 to −0.48) are comparable in magnitude to those reported in prior cross-sectional and observational studies, where correlation coefficients generally ranged between −0.30 and −0.50.

Overall, the findings of the present study align with existing

evidence that lower serum vitamin D levels are associated with increased systemic inflammation, as reflected by elevated CRP, ESR, and IL-6 levels, reinforcing the biochemical link between hypovitaminosis D and inflammatory processes.

Conclusion

The present study was conducted to evaluate vitamin D status and its association with inflammatory markers from a biochemical perspective. The findings demonstrate that vitamin D deficiency was prevalent among the study population and showed a statistically significant inverse relationship with key inflammatory markers. There was a moderate negative connection between serum vitamin D levels and C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and interleukin-6 (IL-6). This means that people with lower vitamin D levels tended to have higher levels of systemic inflammation.

These results support the hypothesis that vitamin D plays an important immunomodulatory and anti-inflammatory role. The observed associations suggest that hypovitaminosis D may contribute to heightened inflammatory activity and could be a potential biochemical indicator of systemic inflammatory status. Therefore, assessment of vitamin D levels may be useful in individuals with elevated inflammatory markers, and correction of deficiency could have beneficial implications in reducing inflammatory burden.

In conclusion, the present study establishes a significant inverse association between serum vitamin D levels and inflammatory markers, highlighting the importance of maintaining adequate vitamin D status in mitigating systemic inflammation and promoting overall health.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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