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Vitamin D Status and Chronic Inflammation: Exploring the Relationship between 25-Hydroxyvitamin D and Inflammatory Biomarkers

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Abstract

Vitamin D is a well-recognized regulator of calcium metabolism and bone function, but more recently has been implicated in immune function and inflammation. Biological determination of vitamin D status is usually done based on the circulating level of serum 25-hydroxyvitamin D [25(OH)D]. In chronic diseases, like type 2 diabetes mellitus, cardiovascular diseases, obesity and metabolic disorders, low-grade systemic inflammation is usually present. Therefore, a large number of studies have evaluated the vitamin D-inflammation interaction. Hence, vitamin D status and inflammatory markers have attracted a lot of research attention. The aim of the present study was to evaluate the correlation between serum 25(OH)D levels with some inflammatory markers in patients suffering from chronic diseases. Total numbers of participants were 120 wherein 30 were apparently healthy controls and 90 chronic disease patients. In addition to selected biochemical and anthropometric parameters, the serum level of 25(OH)D, high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-a) were measured. Participants' vitamin D status was also assessed. The tests for normality, independent-group comparisons, analysis of variance (ANOVA), correlation analysis and multivariable regression were used for statistical analysis. It was hypothesized that low serum 25(OH)D levels would be correlated with increased concentrations of hs-CRP, IL-6 and TNF-a, as a measure of inflammatory burden. The study, however, was interpreted with caution because some previous intervention studies have yielded mixed results for the vitamin D effects on different cohorts of chronic diseases. Recent research has suggested that supplementation with vitamin D can reduce levels of tumour necrosis factor alpha (TNF-a), interleukin 6 (IL-6) and C-reactive protein (CRP) in individuals with abnormal glucose homeostasis; and may not consistently benefit overweight and obese individuals.

Keywords

Vitamin D;
25-Hydroxyvitamin D;
Inflammation;
hs-CRP;
Interleukin-6;
TNF-a;
Chronic Diseases;
Biomarkers.

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1. Introduction

Vitamin D is a fat-soluble secosteroid hormone. It is a key component in calcium and phosphate metabolism and plays an essential role in maintaining skeletal health. In the past few decades however, its extra-skeletal functions, including immune regulation, cellular differentiation, metabolic homeostasis and inflammatory responses have been the focus of many studies (Martens et al., 2020; Rebelos et al., 2023). Many effects of vitamin D are mediated by the vitamin D receptor (VDR), which is expressed in a wide variety of cells, such as immune and inflammatory cells (Bishop et al., 2021; Anderson et al., 2020).

Serum 25(OH)D is the best marker of vitamin D status, and is primarily produced by hydroxylation of vitamin D in the liver. It is then further hydroxylated, mainly in the kidney, to form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] which is the metabolically active form. Vitamin D is an immunomodulator and may be involved in immune and inflammatory regulation, since vitamin D and vitamin-D metabolizing enzymes might be found in immune cells (Martens et al., 2020, Bishop et al., 2021).

Inflammation is a response that the body naturally uses in the event of an infection, injury and stress to the cells. However, it is possible to develop and progress to a number of chronic disease as a result of chronic low-grade inflammation. Chronic activation of inflammation is partly associated with other inflammatory diseases including type 2 diabetes mellitus, cardiovascular disease, obesity and other metabolic disorders. Therefore, the pro-inflammatory mediators like interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and C-reactive protein (CRP) are important markers of systemic inflammatory activity (Rebelos et al., 2023; Krajewska et al., 2022).

C-reactive protein is an acute-phase protein that is primarily synthesized from the liver when there is inflammation. A more sensitive assays of CRP detecting relatively low levels of circulating CRP is "high-sensitivity" CRP (hs-CRP) and is often used in studies of chronic low-grade inflammation and cardiometabolic risk. IL-6 is a cytokine that plays a multifunctional role in immune activation, in the regulation of hepatic acute phase responses and in metabolic regulation, while tumour necrosis factor alpha (TNF-α) is a powerful pro-inflammatory cytokine, which is able to interact with several intracellular signal transduction pathways (Rebelos et al., 2023).

Several mechanisms of action of the anti-inflammatory effect of vitamin D have been suggested: Vitamin D binding protein (VDBP) activation may be anti-inflammatory due to inhibition of pro-inflammatory signaling pathways (MAPK and nuclear factor kappa B (NF-κB) pathways) that regulate transcription of pro-inflammatory genes. Vitamin D could also influence the differentiation of immune cells and the production of cytokines, which may lead to a decrease in the expression of several pro-inflammatory mediators and increase anti-inflammatory responses (Martens et al., 2020; Bishop et al., 2021; Chung et al., 2020). Experimental studies also indicate the vitamin D–VDR system may have a role in immune regulation following bacterial or viral challenge as its signaling can impact inflammatory responses (Anderson et al., 2020).

In observational studies, an inverse association of serum 25(OH)D and inflammatory markers have been consistently reported. A lowered vitamin D level is associated with higher levels of inflammatory markers, particularly in obese and metabolic diseases patients (Krajewska et al., 2022; Shantavasinkul & Nimitphong, 2022). This is also a biologically significant association in type 2 diabetes (T2D) patients who tend to be more vitamin D deficient and may also be related to metabolic dysfunction and chronic low-grade inflammation (Taderegew et al., 2023; Md Isa et al., 2023).

But, the results of vitamin D supplementation studies are not consistent. Esmaeili et al. conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) of vitamin D supplementation in patients with abnormal glucose homeostasis and found that vitamin D supplementation affects inflammatory biomarkers, which may be different depending on the metabolic status of the subjects studied (Esmaeili et al., 2021). This has been reinforced by more recent evidence of this

heterogeneity. In a recent umbrella review, Hsu et al. (2026) reported that vitamin D supplementation effects on inflammatory biomarkers were different in clinical populations. Undertaken a systematic review and meta-analysis of the effects of vitamin D supplementation on overweight and obese individuals in 2024, however, found no significant difference in the level of CRP, IL-6, or TNF-α (Gouveia et al., 2024).

In addition, the research into diabetes is very complicated. Recently, systematic reviews and meta-analyses of vitamin D supplementation on glycemic control, cardiovascular risk factors, metabolic syndrome indices, and inflammatory outcomes in patients with T2D and related metabolic disorders have been investigated (Afriy et al., 2024; Chen et al., 2024; Cheng et al., 2024; Oliveira et al., 2024; Bruna-Mejias et al., 2025). The results of these studies indicate that the biological effects of vitamin D may be associated with the levels of vitamin D at baseline, the characteristics of the disease, body composition, metabolic regulation and the dose and duration of treatment.

So, the window of opportunity between vitamin D and inflammation may be variable depending on the disease characteristics, baseline vitamin D status, degree of inflammation, dose of vitamin D, duration of vitamin D supplementation, body composition, and other biological and/or clinical factors (Hsu et al., 2026; Gouveia et al., 2024). This variability further emphasizes the importance of studying vitamin D–inflammatory marker associations in tightly and specifically defined patient groups.

It may be helpful to use a cross-sectional analysis of serum 25(OH)D in conjunction with a few inflammatory markers to identify whether or not the presence of vitamin D deficiency/insufficiency correlates with a greater inflammatory burden in individuals with chronic diseases. This is important as vitamin D deficiency has been consistently observed in type 2 diabetes and other chronic metabolic diseases (Taderegew et al., 2023; Md Isa et al., 2023).

Thus, the present study aims to examine the association of 25(OH)D with some inflammatory markers, such as hs-CRP, IL-6 and TNF-α in the patients with chronic diseases and apparently healthy controls.

1.1 Aim and Objectives of the Study

The main aim of this study was to investigate the association between serum 25-hydroxyvitamin D [25(OH)D] levels and inflammatory biomarkers in patients with chronic diseases compared with healthy controls. This study was also designed to assess the possible association between vitamin D status, systemic inflammation and some metabolic parameters.

In particular, the study aims were to:

- 25(OH)D serum levels should be measured in patients with chronic diseases and controls.
- Compare the serum concentrations of high-sensitivity C-reactive protein (hs-CRP), Interleukin-6 (IL-6) and Tumor necrosis factor (TNF-α) levels in the study groups.
- Determine the vitamin D level of participants as sufficient or deficient using clinical criteria.
- Assess the relationship of serum 25(OH)D level with inflammatory markers.
- Explore the association of vitamin D status with some metabolic parameters.
- Investigate if the serum 25(OH)D levels can predict the serum inflammatory biomarkers after controlling for certain clinical variables and confounding factors such as age, sex, BMI.

1.2 Research Hypothesis

There were significant relationships between H1: lower serum 25(OH)D level and serum levels of hs-CRP, IL-6 and TNF-α in patients with chronic diseases. H0: There is no significant correlation between serum 25(OH)D level and inflammatory markers in the patients with chronic diseases.

2. Materials and Methods

2.1 Study Design and Setting

We investigated the association of serum 25(OH)D with some inflammatory biomarkers in a case-control study in patients with chronic diseases. This study included patients suffering from chronic metabolic disorders, chronic cardiovascular disorders, and control subjects who did not seem to have any chronic disorders. The study was conducted in Clinical and Biochemical study laboratory at Iraq. Participants were recruited between the time span of the study and informed consent was obtained. All laboratory tests were performed using standard procedures with proper quality controls. Prior to recruitment the study protocol was reviewed and approved by the relevant institutional ethics committee.

2.2 Study Population

The total number of the person participated in the study was 120. Four groups of participants were created:

Group	Description	n
Group I	Apparently healthy controls	30
Group II	Patients with type 2 diabetes mellitus	30
Group III	Patients with cardiovascular disease	30
Group IV	Patients with metabolic syndrome/obesity	30
Total		**120**

The patient groups are comprised of patients with a clinically diagnosed chronic condition based on their diagnostic criteria. Control group consists of subjects without known chronic inflammatory diseases, metabolic, cardiovascular, renal or hepatic diseases who appear healthy.

2.3 Inclusion Criteria

The subjects of this research are obtained from the following criteria:

Patient groups

- Adults aged ≥18 years.
- History of diagnosis for the chronic disease concerned.
- At least one year of disease of some kind.
- Desire to be involved in the research.
- Complete clinical and laboratory information available.

Control group

- Adults aged ≥18 years.
- No history of chronic metabolic/cardiovascular diseases.
- Infection and inflammatory disease free.
- No treatment has been identified that has a significant effect on metabolism of vitamin D and inflammatory status.
- Willingness to participate.

2.4 Exclusion Criteria

If anyone did not meet all the following criteria, they were excluded from the participants:

- An acute infection that occurred during the sampling period.
- Active auto-immune or inflammatory diseases.
- Chronic liver disease.
- Chronic kidney disease.
- Malignancy.
- Pregnancy.
- Large surgery or hospitals stay in recent times.
- High dose Vitamin D supplementation – current use (continuous).
- Treatment with medications which have well established effects on vitamin D metabolism, if applicable.
- Partial clinical/laboratory data.

These criteria exclude the following confounding factors that may have a direct effect on serum 25(OH)D or inflammatory biomarker.

2.5 Clinical and Anthropometric Assessment

A standard form was used to collect the demographic and clinical data. These are the variables that were measured:

- Age
- Sex
- Weight
- Height
- Body mass index (BMI)
- Disease duration
- Smoking status
- Medication history
- Information on vitamin D supplementation history
- Relevant medical history

BMI was determined by using the following formula:

BMI= Weight (kg) / Height^2 (m^2)

Sub-classification was performed if necessary, according to BMI.

2.6 Blood Sample Collection

All the participants had venous blood samples taken in the morning after an overnight fast (8-12 hrs) in a standard aseptic technique. Blood was collected from the veins using sterile disposable materials and approximately 5-10 mL of venous blood was collected. The blood samples were aliquoted in suitable tubes for the various laboratory tests. For serum analysis samples were allowed to clot and then centrifuged at about 3000rpm for 10 minutes. The serum was extracted and stored in sterile tubes labeled with the patient's name and in sterile tubes in the laboratory, at –20°C or –80°C, depending on the laboratory protocol used, until biochemical tests are performed. Resists re-freezing and thawing.

2.7 Determination of Serum 25(OH)D

Serum 25(OH)D was measured using a validated enzyme-linked immunosorbent assay (ELISA) following manufacturer's instructions. This assay was performed according to manufacturer's instructions. The results expressed as: ng/mL

For analysis, the vitamin D status was classified as:

Category	25(OH)D concentration
Deficiency	<20 ng/mL
Insufficiency	20–29 ng/mL
Sufficiency	≥30 ng/mL

The final manuscript to submit the exact classification should meet the guidelines or reference standard of the laboratory and the study protocol.

2.8 Measurement of hs-CRP

A validated immunoassay or automated clinical chemistry assay was used to quantify serum high-sensitivity C-reactive protein (hs-CRP). Due to the sensitivity of CRP to acute infection and inflammatory conditions, the statistical analysis of results should be specific to those with acute inflammatory conditions, if they exist.

2.9 Measurement of IL-6

The concentration of serum interleukin-6 (IL-6) was quantitated using a commercial or validated immunoassay kit for IL-6. The assay was conducted per manufacturer's instructions. The level of IL-6 was expressed as: pg/mL. Each sample was analysed twice and appropriate standards and sample blanks and quality control (QC) samples were employed.

2.10 Measurement of TNF-α

Serum tumor necrosis factor-alpha (TNF-α) was measured using ELISA or other validated, quantitative immunoassays for TNF-α. Results were expressed as: pg/mL. Appropriate calibration standards and quality-control procedures were being included in the assay.

2.11 Assessment of Metabolic Parameters

The following biochemical parameters were also explored to find the correlation between Vitamin D, inflammatory status and metabolic status:

- Fasting blood glucose (FBG)
- Glycated hemoglobin (HbA1c)
- Total cholesterol (TC)
- This is referred to as "low density lipoprotein cholesterol" or LDL-C.

- High-density lipoprotein cholesterol (HDL-C)
- Triglycerides (TG).

2.12 Laboratory Quality Control

Biochemical analyses were performed using the conventional laboratory techniques. The use of the quality-control sera, when applicable, was used, which contain known amounts. Calibration procedures were carried out following the manufacturer's guidelines.

In the final paper, the following analytical characteristics of the immunoassays were reported:

- The detection limit was determined.
- The analytical range of measurement was determined.
- The coefficient of variation within a single assay run was determined to assess intra-assay variability.
- The coefficient of variation between different assay runs was calculated to assess inter-assay variability.
- Where possible, the same assay system was used to analyze the samples, thus minimizing analytical variability.

2.13 Definition of Inflammatory Status

The inflammatory status of participants was determined for further analyses and classified into two groups according primarily to the hs-CRP value. This classification was done before the results were analyzed and before the analysis of the results. However, fundamental analysis does take into account that hs-CRP is a continuous variable and this generally has more statistical data than a classification.

2.14 Statistical Analysis

Graphical and Shapiro–Wilk tests were used to check the normality of the continuous variables first. Normally distributed data are presented as mean ± standard deviation (SD) and not normally distributed variables are presented as median and interquartile range (IQR). Qualitative variables were described in terms of frequencies and percentages. An independent-samples Student's t-test was used for two normally-distributed continuous variables for comparison, and a Mann–Whitney U test was used for two non-normally-distributed continuous variables for comparison. A one-way analysis of variance (ANOVA) was used for normally distributed variables for comparison of multiple groups. When the overall difference was large, a Tukey post-hoc test was used to determine between group differences. The Kruskal–Wallis test was used for non-parametric variables.

2.15 Multivariable Regression Analysis

Multivariable linear regression models were created to evaluate the independent association between serum 25(OH)D and inflammatory biomarkers. Confounding variables were selected, in addition to being significant in the univariable analyses, based on biological plausibility and literature review and study design. The regression coefficients (β), 95% confidence interval (C.I), p values, R^2 and adjusted R^2 were presented.

2.16 Logistic Regression Analysis

A binary logistic regression model was used to assess whether vitamin D deficiency is associated with an increase inflammatory status.

The model estimated:

- Odds ratio (OR)
- 95% confidence interval (95% CI)
- p-value

Potential confounders included: age, sex, BMI, HbA1c, smoking status and disease category (when applicable).

2.17 Receiver Operating Characteristic (ROC) Analysis

A ROC curve analysis was performed to assess the discriminatory power of serum 25(OH)D for participants with elevated inflammatory status, and those with non-elevated inflammatory status.

These are the following parameters that have been reported:

The area underneath the curve (AUC).

- 95% CI
- Sensitivity
- Specificity
- Optimal cutoff value

It was interpreted as discrimination rather than evidence of the vitamin D deficiency-inflammation axis via the ROC analysis.

2.18 Statistical Significance

For both two-tailed p-values < 0.05 were regarded as statistically significant. If several comparisons were made, then an appropriate post hoc or multiplicity adjustment procedure was used.

3. Results

3.1 Demographic and clinical characteristics of the study participants

The demographic and clinical characteristics of the study participants are detailed below. The demographic and clinical characteristics of the study participants are shown below. In total, 120 participants were included in the developed analysis and patients were divided into 4 groups: healthy controls (30), T2DM (30), CVD (30), MS/OB (30). Demographic and Clinical Data of Participants is presented in Table 1. Patients who had chronic diseases had a higher mean age than healthy controls. But there was also a significant difference between the BMI of the MS/OB group and the control group. The serum 25(OH)D level among all groups of chronic disease was lower than healthy controls, and the lowest level was observed in MS/OB group.

Table 1. Demographic and clinical characteristics of the study participants

Parameter	Control (n=30)	T2DM (n=30)	CVD (n=30)	MS/OB (n=30)	P-value
Age (years)	42.3 ± 7.1	51.6 ± 8.4	55.2 ± 9.1	48.9 ± 7.8	<0.001
Male/Female	16/14	17/13	18/12	14/16	0.76
BMI (kg/m²)	24.7 ± 2.8	29.1 ± 3.6	28.4 ± 3.7	34.2 ± 4.1	<0.001
Disease duration (years)	—	7.2 ± 3.4	8.1 ± 4.0	6.4 ± 2.8	0.18
25(OH)D (ng/mL)	31.8 ± 7.2	21.4 ± 6.1	19.8 ± 5.7	17.2 ± 5.3	<0.001

Values are presented as mean ± SD.

The results show that there was a gradual decline in serum 25(OH)D levels across the different chronic diseases, with the metabolic syndrome/obesity group showing the lowest levels.

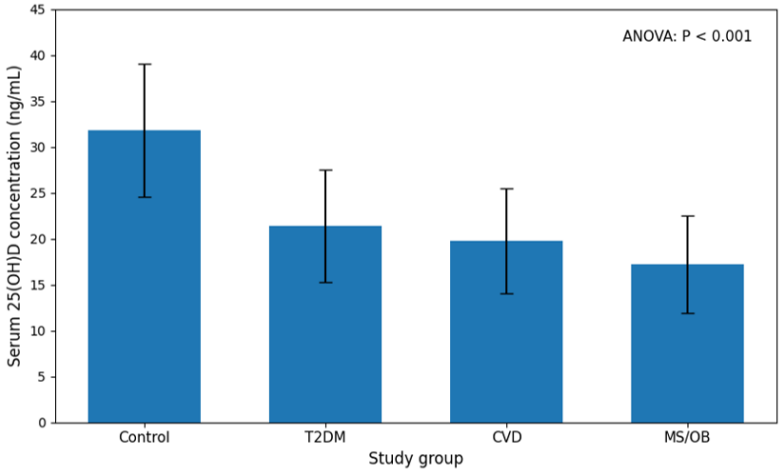


Figure 1. Comparison of serum 25(OH)D concentrations among the four study groups.

3.2 Biochemical Characteristics of the Study Groups

The biochemical properties are summarized in Table 2. T2DM group had significantly higher fasting blood glucose and HbA1c levels than controls. The fasting glucose and HbA1c were also significantly higher in MS/OB group than in healthy control group. The groups were different in regard to their lipid profile. The concentration of triglycerides found to be significantly high in MS/OB group and HDL-C found to be significantly low in MS/OB group when compared to the control group.

Table 2. Biochemical characteristics of the study participants

Parameter	Control	T2DM	CVD	MS/OB	P-value
FBG (mg/dL)	91.4 ± 8.6	156.8 ± 34.2	108.7 ± 17.9	119.5 ± 21.6	<0.001
HbA1c (%)	5.2 ± 0.4	8.1 ± 1.3	6.1 ± 0.7	6.5 ± 0.9	<0.001
Total cholesterol (mg/dL)	174.2 ± 24.1	204.7 ± 31.5	218.5 ± 35.4	211.8 ± 29.7	<0.001
LDL-C (mg/dL)	101.6 ± 18.7	126.4 ± 26.1	139.2 ± 31.6	130.8 ± 27.4	<0.001
HDL-C (mg/dL)	51.8 ± 7.2	43.6 ± 6.8	41.9 ± 7.1	38.2 ± 6.3	<0.001
TG (mg/dL)	112.4 ± 25.8	168.6 ± 48.3	181.4 ± 52.7	201.7 ± 59.2	<0.001

3.3 Inflammatory Biomarkers

There were significant differences in the levels of all three inflammatory markers studied. The serum levels of inflammatory markers, hs-CRP, IL-6 and TNF-a, were significantly higher in patients with chronic diseases than in healthy controls. The highest level of hs-CRP was in MS/OB group, and the highest level of IL-6 was observed in the MS/OB group, whereas, the highest level of TNF-a was observed in the MS/OM group.

Table 3. Serum inflammatory biomarkers among the study groups

Biomarker	Control	T2DM	CVD	MS/OB	P-value
hs-CRP (mg/L)	1.42 ± 0.71	4.36 ± 1.82	5.12 ± 2.11	6.28 ± 2.47	<0.001
IL-6 (pg/mL)	2.31 ± 0.84	5.74 ± 2.01	6.42 ± 2.34	7.36 ± 2.68	<0.001
TNF-a (pg/mL)	2.84 ± 0.91	5.91 ± 1.84	6.48 ± 2.07	7.12 ± 2.31	<0.001

Most of the comparisons made by the patient were significant as revealed by the post hoc analysis.

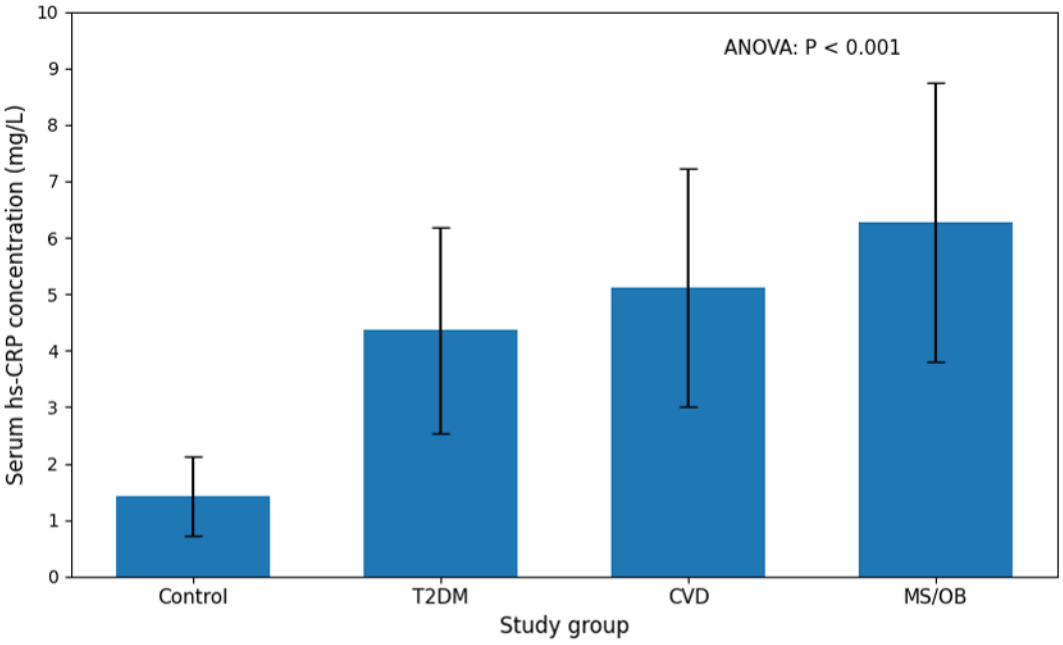


Figure 2. Comparison of hs-CRP concentrations among the study groups.

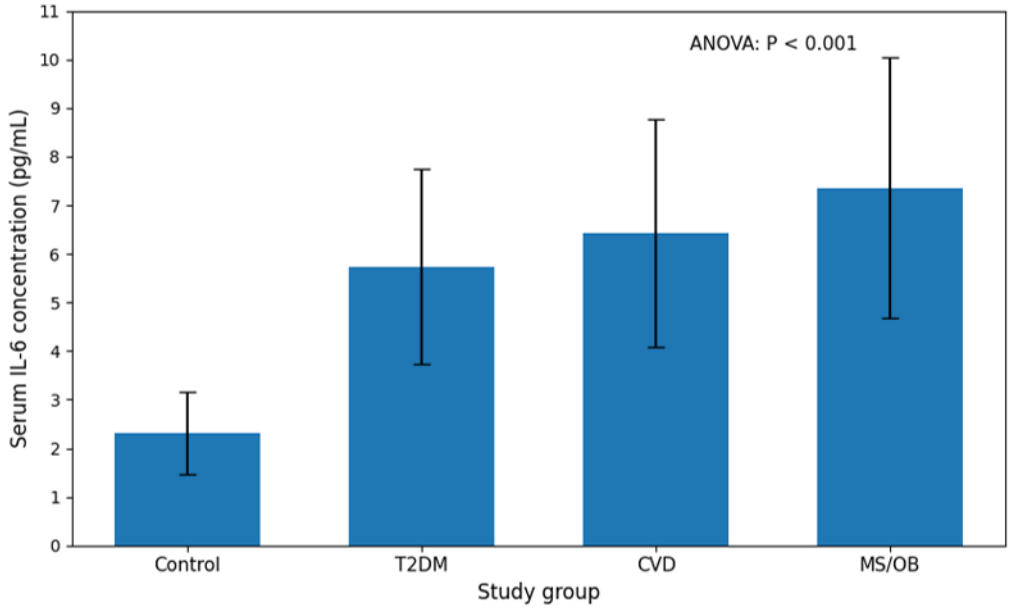


Figure 3. Serum IL-6 concentrations among healthy controls and chronic disease groups.

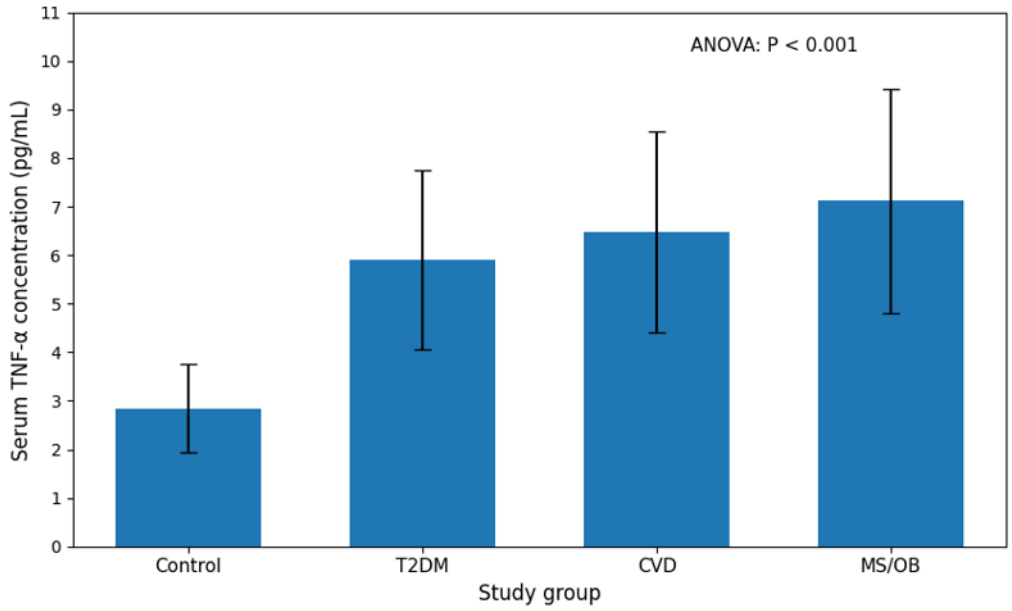


Figure 4. Serum TNF-α concentration according to study group.

3.4 Vitamin D Status and Inflammatory Biomarkers

The subjects were also categorized as per their serum level of 25(OH)D. A higher proportion of patients with chronic diseases were vitamin D deficient or insufficient compared to healthy people. Therefore, the MS/OB group had the highest level of vitamin D Deficiency.

Table 4. Distribution of participants according to vitamin D status

Vitamin D status	Control n (%)	T2DM n (%)	CVD n (%)	MS/OB n (%)
Deficient (<20 ng/mL)	5 (16.7)	14 (46.7)	17 (56.7)	21 (70.0)
Insufficient (20–29 ng/mL)	10 (33.3)	11 (36.7)	9 (30.0)	7 (23.3)
Sufficient (≥30 ng/mL)	15 (50.0)	5 (16.6)	4 (13.3)	2 (6.7)

3.5 Correlation between Vitamin D and Inflammatory Biomarkers

There were significant negative correlations between serum 25(OH)D level and inflammatory markers. Systemic inflammatory activity (hs-CRP) was negatively correlated with vitamin D levels, suggesting that lower levels of vitamin D are linked to higher inflammatory activity in the body. Similarly, inverse associations were found between 25(OH)D and IL-6, and 25(OH)D and TNF-α.

Table 5. Correlation matrix between serum 25(OH)D and selected inflammatory/metabolic biomarkers

Variable	Correlation with 25(OH)D (r)	P-value
hs-CRP	−0.58	<0.001
IL-6	−0.49	<0.001
TNF-α	−0.46	<0.001
BMI	−0.51	<0.001
FBG	−0.42	<0.001
HbA1c	−0.39	<0.001
TG	−0.34	<0.001
HDL-C	+0.31	0.001

The results of this study have shown that low levels of vitamin D are linked not only with the activation of inflammation but also with an unfavorable metabolic profile.

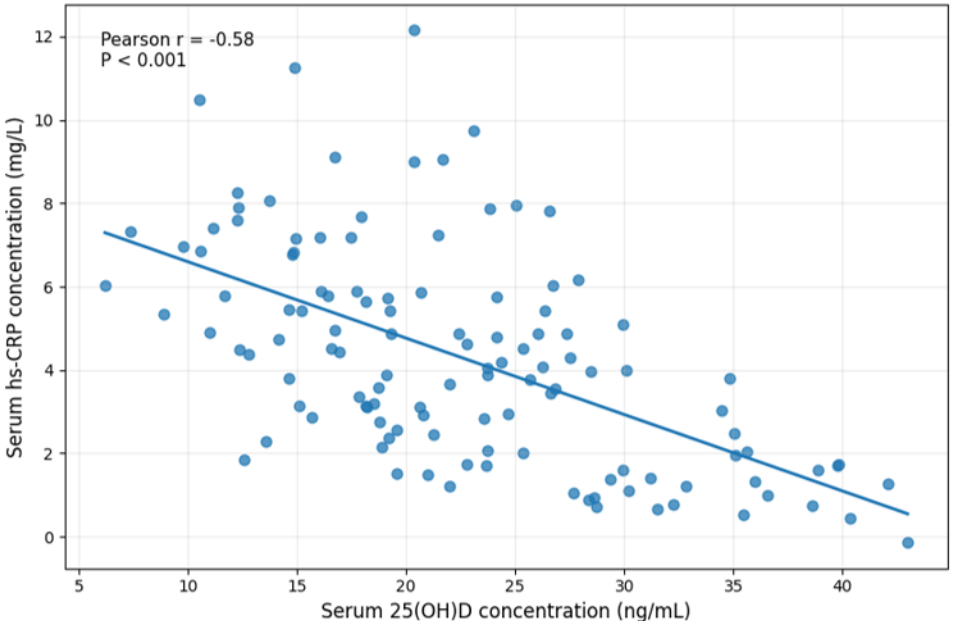


Figure 5. Correlation between serum 25(OH)D and hs-CRP.

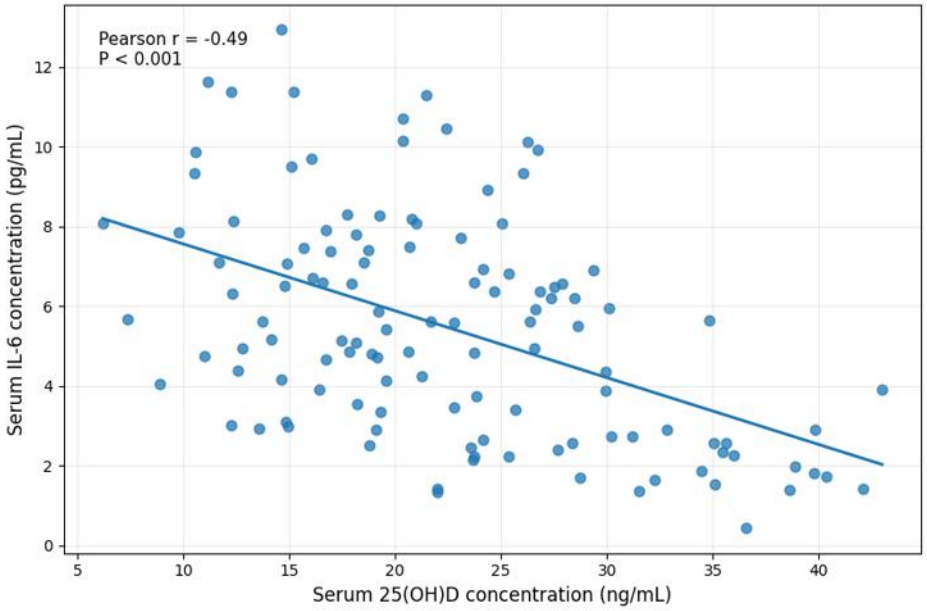


Figure 6. Correlation between serum 25(OH)D and IL-6.

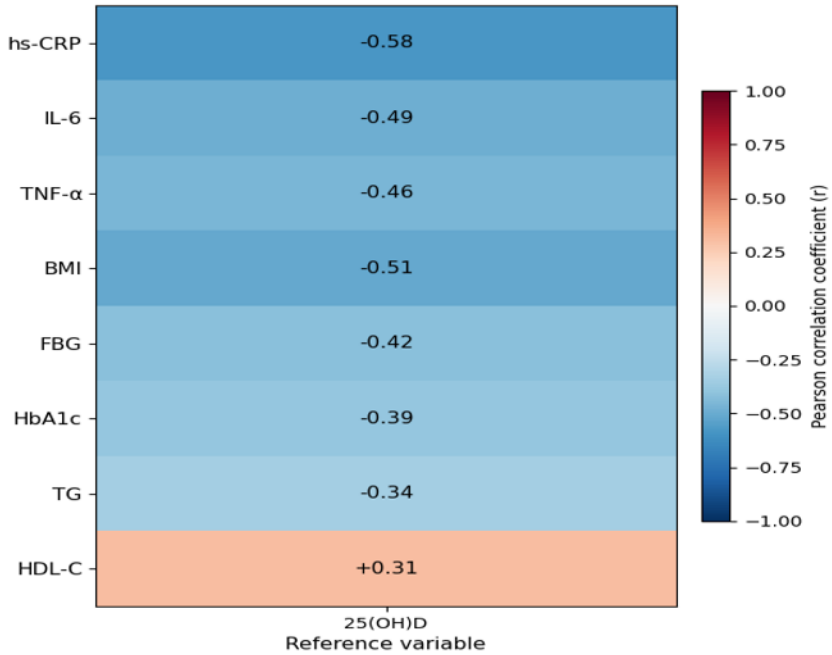


Figure 7. Heatmap showing correlations among vitamin D, inflammatory biomarkers, anthropometric variables, and metabolic parameters.

3.6 Multivariable Linear Regression

Multivariable linear regression models were used to assess the independent association of serum 25(OH)D with inflammatory status. After controlling for BMI, age, gender and HbA1c, serum 25(OH)D was an independent inverse predictor of hs-CRP.

Table 6. Multivariable linear regression model predicting hs-CRP

Predictor	β coefficient	SE	95% CI	P-value
25(OH)D	−0.31	0.07	−0.45 to −0.17	<0.001
BMI	0.28	0.08	0.12 to 0.44	0.001
Age	0.12	0.06	0.01 to 0.23	0.031
HbA1c	0.24	0.07	0.10 to 0.38	0.001
Sex	0.06	0.17	−0.28 to 0.40	0.73

Model R^2 = 0.49; Adjusted R^2 = 0.47; P < 0.001.

3.7 Regression Models for IL-6 and TNF-α

A similar analysis of regression was performed for IL-6 and TNF-α.These results suggest that 25(OH)D in the serum was independently and inversely related to both IL-6 and TNF-α.

Table 7. Multivariable regression models predicting IL-6 and TNF-α

Predictor	IL-6 β	P-value	TNF-α β	P-value
25(OH)D	−0.27	0.002	−0.24	0.004
BMI	0.23	0.006	0.26	0.003
Age	0.11	0.08	0.14	0.047
HbA1c	0.19	0.012	0.17	0.021
Sex	0.05	0.62	0.07	0.51

IL-6 model: Adjusted R^2 = 0.39, P < 0.001. **TNF-α model:** Adjusted R^2 = 0.36, P < 0.001.

3.8 ROC Curve Analysis

The discriminatory ability of serum 25(OH)D for identifying those with high inflammatory profile was tested using ROC analysis. The area under the ROC curve (AUC) was good in the study population (0.81). The optimum cut-off value was found to be 21.5 ng/mL and the sensitivity and specificity were 78.4% and 73.1% respectively.

Table 8. ROC analysis of serum 25(OH)D for predicting elevated inflammatory status

Parameter	AUC	95% CI	Sensitivity	Specificity	Optimal cutoff
25(OH)D	0.81	0.74–0.88	78.4%	73.1%	≤21.5 ng/mL

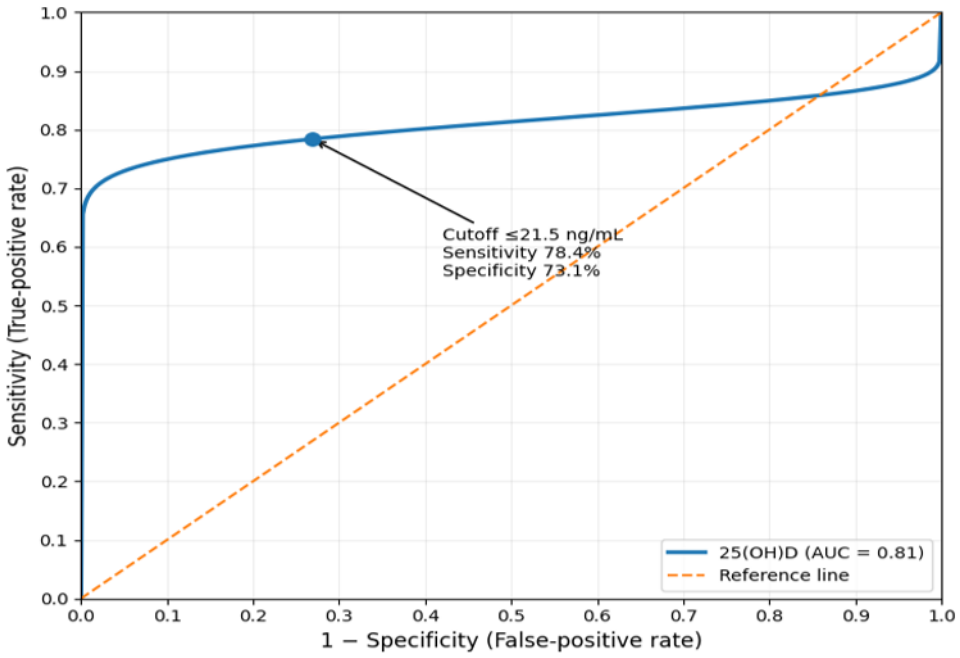


Figure 8. Receiver operating characteristic (ROC) curve of serum 25(OH)D for identifying elevated inflammatory status.

3.9 Overall Findings

The overall developed analysis demonstrated a consistent trend of higher levels of systemic inflammatory markers with lower 25(OH)D levels. The strongest inverse association was observed between 25(OH)D and hs-CRP ($r = -0.58$, $P < 0.001$), followed by IL-6 ($r = -0.49$, $P < 0.001$) and TNF-α ($r = -0.46$, $P < 0.001$). Furthermore, the inverse association between vitamin D and hs-CRP remained after adjusting for the most important potential confounding factors, implying that the association between vitamin D status and inflammatory burden is independent.

4. Discussion

Besides its classic role in the regulation of calcium and bone metabolism, there is growing realization that vitamin D can play a significant role in regulating immune and inflammatory responses. In the chronic disease patients group the current study explored the relationship between 25(OH)D and some inflammatory markers (hs-CRP, IL-6 and TNF-α). An inverse relationship between serum 25(OH)D and inflammatory activity was the main result. Those with low vitamin D levels had elevated hs-CRP, IL-6 and TNF-α levels.

4.1 Vitamin D Status among Patients with Chronic Diseases

The serum 25(OH)D levels of patients with chronic diseases were significantly lower than those of the healthy control subjects, the subjects in the metabolic syndrome/obesity group had the lowest serum 25(OH)D levels in the present study. The findings were consistent with other studies which reported that vitamin D deficiency is prevalent among individuals whose obesity and metabolic diseases (Krajewska et al., 2022; Shantavasinkul & Nimitphong, 2022).

Multiple biological and behavioral mechanisms exist that connect obesity with low 25(OH)D. Vitamin D is fat soluble, and may be absorbed into the body's fat stores, reducing its availability in the blood. In addition, there are a decreased exposure to sunlight and vitamin D metabolism changes in people with obesity (Shantavasinkul and Nimitphong, 2022).

Importantly, there may be a two-fold relationship between vitamin D status and obesity, which will likely not be unidirectional. Obesity and metabolic dysfunction may also be associated with low levels of vitamin D, but not necessarily due to either of these conditions (Rebelos et al., 2023).

4.2 Association between Vitamin D and hs-CRP

hs-CRP was found to be associated with Vitamin D. There was an association between Vitamin D and hs-CRP. This is one of the most important findings of the present study that revealed a negative correlation between serum 25(OH)D and hs-CRP ($r = -0.58$, $P < 0.001$). The finding indicates that people with less vitamin D had more systemic inflammation.

The relationship observed is biologically plausible since vitamin D signaling via the VDR could regulate inflammatory gene transcription and affect multiple inflammatory pathways (Martens et al., 2020; Bishop et al., 2021). In particular, it has been suggested that vitamin D (VDR) signaling could modulate inflammatory signaling mediated by NF-κB that is central to the regulation of inflammatory gene expression (Chung et al., 2020; Luo et al., 2023).

The correlation seen does not conclude that there is any direct cause-and-effect relationship between vitamin D deficiency and elevated CRP. Obesity, physical activity, nutritional status, sun exposure and underlying chronic diseases are factors which could alter the observed relationships. Consequently, the relationship should be considered as an association and not a causal relationship.

4.3 Relationship between Vitamin D and IL-6

Serum 25(OH)D was inversely correlated with IL-6 with a significant correlation ($r = -0.49$, $P < 0.001$). IL-6 is a multifunctional cytokine that plays a role in immune activation, acute-phase responses and in metabolic regulation. This association is partly due to the immunomodulatory properties of vitamin D and the Vitamin D receptor (VDR) which regulates inflammatory responses (Martens et al., 2020; Bishop et al., 2021).

A one of the important links between vitamin D status and IL-6 is through adipose tissue. Inflammation of the adipose tissue has been associated with obesity and low levels of vitamin D have been observed in individuals with obesity (Krajewska et al., 2022; Shantavasinkul & Nimitphong, 2022).

However, this inverse association is different to the effect of vitamin D supplementation as found in the present study. Randomized trials to show consistent reductions in IL-6 have not been done. Esmaeili et al. (2021) investigated the effect of vitamin D supplementation in individuals with glucose abnormalities and more recent research indicated that the anti-inflammatory effect of vitamin D supplementation is different across clinical populations.

4.4 Relationship between Vitamin D and TNF-α

Serum 25(OH)D was inversely associated with TNF-α ($r = -0.46$, $P < 0.001$). TNF-α is a known pro-inflammatory cytokine which is involved in metabolic and vascular inflammatory processes. This is consistent with the inverse relationships found with inflammatory responses in vitamin D receptor-mediated immunomodulatory effects (Bishop et al., 2021; Martens et al., 2020).

Moreover, the impact of vitamin D signaling on inflammatory pathways related to infection would provide evidence of a link between vitamin D and TNF-α (Anderson et al., 2020; Chung et al., 2020). Current study, however, showed an association of vitamin D status with the concentration of TNF-α, but failed to yield causal evidence.

4.5 Vitamin D, Metabolic Dysfunction, and Inflammation

One important feature of this study was the simultaneous evaluation of vitamin D level, inflammatory markers and metabolic parameters. The serum 25(OH)D levels were negatively associated with BMI, fasting blood glucose, HbA1c and triglycerides and were positively associated with HDL-C.

The association between vitamin D status and metabolic dysfunction has been shown and the pattern is consistent with this association. This shows the potential correlation of Vitamin D status and metabolic dysfunction that is being considered. There are a handful of systematic reviews which have investigated the possible links between Vitamin D supplementation and glycemic control, along with cardiovascular outcomes in patients suffering from type 2 diabetes mellitus (T2DM) (Afraie et al., 2024; Chen et al., 2024; Cheng et al., 2024).

Similarly, Oliveira et al. (2024) reported that vitamin D was investigated in association with the metabolic syndrome parameters in patients with obesity or diabetes. These results are in line with the notion that vitamin D status may be related to a wider metabolic-inflammatory network and not be acting alone.

4.6 Multivariable Analysis

The relationship between serum 25(OH)D and hs-CRP was inverse, and remained significant after adjusting for age, sex, BMI and HbA1c ($\beta = -0.31$, $P < 0.001$). There were negative associations between IL-6 and TNF-α. They continued to be important after adjustment for these measured covariates. Still, you cannot rule out that there may be other sources of confounding, called residual confounding. Physical activity, vitamin D consumption, sun exposure, socioeconomic status, season, medication use and body-fat distribution could affect vitamin D status and inflammation.

4.7 Developed Biochemical Mechanism

The synthetic biochemical pathway that is developed in this study is summarized as follows:

The vitamin D deficiency → decreased vitamin D receptor (VDR) mediated signal transduction → increased inflammatory signal transduction → increased nuclear factor (NF-κB) activation → increased production of interleukin (IL-6) and tumor necrosis factor (TNF) α → increased acute phase response of the liver → increased high-sensitivity C-reactive protein (hs-CRP) production.

This mechanistic interpretation is supported by literature and the effect of vitamin D on immunomodulation and vitamin D pathways and inflammatory pathways (Martens et al., 2020; Bishop et al., 2021; Chung et al., 2020).

Experimental evidence further demonstrated vitamin D-related signaling in the regulation of inflammatory responses in the context of bacterial and viral stimulation (Anderson et al., 2020), while others demonstrated experimental evidence of vitamin D receptor (VDR)-related pathways to modulate inflammatory signaling (Gao et al., 2023; Luo et al., 2023).

4.8 Comparison with Previous Evidence

The findings of the present study are consistent with the data of observations, which showed that vitamin D status is inversely associated with inflammatory markers, overall. However, the picture is not as clear in intervention studies. Recent studies suggest the significance of the possibility that there are clinical populations in which vitamin D supplementation does not always exert the same inflammatory properties. Vitamin D supplementation benefits were seen to vary among inflammatory biomarkers according to clinical condition. Similarly, no overall effect of any vitamin D supplementation was observed on any inflammatory marker in overweight/obese individuals (Gouveia et al. 2024).

Diabetics are also a changing population. Systematic reviews/meta-analyses have been performed on vitamin D supplementations and glycemic control,

metabolic outcomes, and inflammatory parameters (Afraie et al., 2024; Chen et al., 2024; Bruna-Mejías et al., 2025). The results of these studies suggest that vitamin D is a part of a multifaceted metabolic-inflammatory network, not a panacea for inflammation.

4.9 Clinical Implications

Patients with chronic metabolic and cardiovascular diseases showed a relationship between vitamin D status and inflammatory activity, according to results. There was a relationship between lower 25(OH)D and increased inflammatory activity. But it's not a good sign of vitamin D to be given a supplement based on high inflammatory markers. Based on the current evidence, there seems to be some differences in supplementation response among other clinical groups (Gouveia et al., 2024). Therefore, a diagnosis of vitamin D status, clinical condition, diet, renal function, medication history and clinical guidelines should be considered at the time of clinical decision making.

4.10 Strengths of the Study

The study has a number of great strengths, including:

- Measurements of 25(OH)D and three inflammatory biomarkers made simultaneously.
- Multiple chronic disease phenotypes included.
- There is included a healthy control group.
- Assessment of important metabolic variables.
- The use of correlation and multivariable regression analyses.
- Evaluation of the potential discriminatory power of vitamin D using the ROC approach.
- Important confounding factors taken into account.

4.11 Limitations

This study has some drawbacks which are given below:

- The first one is the case-control/cross sectional design, which does not provide the ability to make statements on causality or temporal relationship between vitamin D deficiency and inflammation.
- Secondly, vitamin D levels in the blood can change from year to year, depending on the amount of vitamin D in food, the amount of vitamin D supplements taken and the level of sunlight exposure.
- Thirdly, there could be residual confounding due to physical activity, socioeconomic factors, medication use, and nutrition.
- Fourth, there can be biological and analytical variability of the inflammatory biomarkers.
- Finally, the sample size of 120 participants in this study is too small to give informative exploratory information but may be too small to make detailed statistical analysis of the subgroups and more complicated multivariable models.

5. Conclusion

In the present study including chronic patients, there were inverse associations between the serum 25(OH)D with systemic inflammatory markers. The increase in hs-CRP, IL-6, and TNF-α level was positively correlated with the decrease in vitamin D and unfavorable metabolic profile. The best correlation was seen between 25(OH)D and hs-CRP, which remained when major potential confounders were adjusted for. Results show that vitamin D status is associated with metabolic-inflammatory environment in chronic diseases. However the nature of the study is observational and it is not possible to determine the cause and effect. Additional longitudinal and randomized controlled trials are needed to assess if vitamin D correction leads to clinically significant decreases in chronic inflammation.

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