



Assessment of Chemokines and Coagulating Factors in Sample of Iraqi Patient with Vitamin D3 Deficiency



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Abstract

Background: Vitamin D3 deficiency is associated with immune dysregulation, increased chemokine expression, and alterations in coagulation, indicating its role in inflammatory and hemostatic imbalance. **Objective:** The purpose of the present study was to evaluate the effects of acute vitamin D3 deficiency on chemokines C-C motif ligand 2 (CCL2), C-X-C motif chemokine ligand 12 (CXCL12), and coagulation factors, including prothrombin (PT), fibrinogen (FG), and antihemophilic factor (FVIII) in women. **Methodology:** This case-control study was conducted in the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, from January 2025 to September 2025. It comprised 60 female patients with vitamin D3 deficiency. Vitamin D3, CCL2, CXCL12 using enzyme-linked immunosorbent assay ELISA and C-reactive protein (CRP) serum levels by sandwich immunodetection method were determined, as well as PT, FG, and FVIII also by ELISA, and complete blood count (CBC) by electrical impedance and colorimetric method to detect parameters. **Results:** The results showed significantly elevated levels of CCL2, CXCL12, and CRP in patients with severe vitamin D3 deficiency compared with healthy controls, whereas no notable differences were observed in complete blood count parameters. In terms of coagulation factors, patients exhibited reduced PT levels along with increased FVIII activity and FG concentrations. Correlation analysis revealed an inverse association between vitamin D3 status and CCL2 in the patient group. **Conclusion:** Our study has shown a link to inflammatory chemokines, CCL2, and coagulation factor changes without hematological changes. The negative correlation between vitamin D3 and CCL2 highlights that vitamins have immunomodulatory effects and may relate to secondary imbalance of hemostasis. [Bangladesh Journal of Infectious Diseases, June 2026;13(1):126-136]

Keywords: Vitamin D3; CCL2; CXCL12; prothrombin, fibrinogen; antihemophilic factor

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Introduction

Vitamin D, comprising vitamin D2 and vitamin D3, is a secosteroid vitamin found in organisms. Photochemical processes yield vitamin D3, where 7-dehydrocholesterol reacts with ultraviolet B (UVB) radiation with an optimal reaction at 295 nm, causing the B ring to open, resulting in pre-vitamin D3. It is a thermolabile transient intermediate which then is oxidized to vitamin D3 through a

conformational isomerization that depends on heat. Exposing pre-vitamin D3 to increased amounts of UVB radiation may allow conversion of pre-vitamin D3 into other substances, including lumisterol and tachysterol¹. For many years, vitamin D3 was considered the only biologically relevant prohormone, whereas lumisterol, tachysterol, and other related derivatives were thought to be biologically inactive or merely degradation

by-products². In addition to controlling blood levels of calcium and phosphorus, vitamin D3 is essential for the growth and upkeep of the skeletal system³. Along with its classical action, vitamin D has a number of other biological effects, such as a protective effect against some cancers, controlling inflammatory reactions, and decreased risk of disorders, including type II diabetes and multiple sclerosis⁴.

Serum 25-hydroxyvitamin D3 (25-OH-D3) is utilized to assess the status of blood vitamin D3 levels. International guidelines conclude that levels of 30 ng/mL and above are said to be adequate, those of 20 to 29 ng/mL are said to be inadequate, and the level of 19 ng/mL and below is said to indicate a deficiency of vitamin D⁵. A severe deficit of vitamin D is characterized by a level below 12 ng/mL⁶. The amount of vitamin D in the human body is influenced by many variables and circumstances, including solar exposure, skin color, latitude, season, clothing type, nutrition, and vitamin D supplementation⁷.

Vitamin D is an oil-soluble vitamin; thus, there may be toxicity, although it is quite rare. Hypervitaminosis D is normally caused by overdose of oral supplements and not long exposure to sunlight. Toxicity is said to occur at serum 25-hydroxyvitamin D levels above 88 ng/mL. As a consequence of acute toxicity, hypercalcemia can follow, the symptoms of which include confusion, loss of appetite, vomiting, increased urination and thirst, and muscle weakness. The toxicity may lead to complications in the long-term, such as nephrocalcinosis and pain in the bone⁶. Reduced endogenous production is also the etiology of vitamin D3 deficiency. Dysfunction of 25-hydroxylation is also seen in individuals with chronic liver disease, including cirrhosis, and defects in 1 α hydroxylation are apparent in renal failure and hypoparathyroidism⁸.

Skin pigmentation is very important in cutaneous synthesis of vitamin D3. The higher the melanin concentration of the darker skin, it serves as a natural filter to UVB radiation, decreasing the transformation of 7-dehydrocholesterol to vitamin D3. Consequently, individuals with darker skin exhibit reduced efficiency in the cutaneous synthesis of vitamin D3 and are therefore more susceptible to vitamin D3 deficiency, even under conditions of normal sunlight exposure⁹. Vitamin D3 is essential for maintaining calcium and phosphate homeostasis by enhancing their intestinal absorption. It plays a critical role in bone mineralization and is involved in key physiological

processes, including muscle contraction, nerve conduction, and the prevention of hypocalcemia¹⁰. Vitamin D3 deficiency degrades calcium assimilation, favors secondary hyperparathyroidism/bone resorption, and raises the chance of a reduced mineral density of the bones and bone illnesses like osteomalacia or osteoporosis¹¹. The main natural factor that influences the production of vitamin D3 in the skin is sunlight exposure, where its efficiency varies with the intensity of UVB, the solar angle, duration of exposure, and geographic region. Poor or insufficient exposure to sun significantly decreases cutaneous production of vitamin D3, especially in groups that need more UVB irradiation like those with darker skin coloration¹². General fatigue and lack of energy: vitamin D3-deficient people tend to feel constantly tired regardless of whether they get enough sleep. This fatigue may result from systemic effects of low calcium levels and impaired muscle and metabolic function¹³. Reduced physical performance, lower physical performance and increased risk of falls, particularly in older adults with severe deficiency, have been described in recent clinical reviews¹⁴.

Vitamin D deficiency has been consistently linked to an elevated susceptibility to infections. While recent interventional studies on vitamin D3 supplementation have yielded variable outcomes, observational research repeatedly demonstrates that individuals with low vitamin D status are at a higher risk of developing infections, particularly those affecting the respiratory system¹⁵.

Chemokines are a class of small signaling proteins composed of a single polypeptide chain, typically ranging from 67 to 127 amino acids in length. They are structurally classified based on the arrangement of conserved cysteine residues near the N-terminal region into major subfamilies, including CXC, CC, and CX3C chemokines. In CC chemokines, the first two cysteine residues are positioned adjacent to each other, whereas in CXC chemokines, they are separated by a single amino acid. Functionally, chemokines play a central role in regulating leukocyte trafficking, thereby contributing to immune surveillance, homeostasis, and both innate and adaptive immune responses¹⁶. Chemokine C-C motif ligand 2, or monocyte chemotactic protein 1 (MCP-1), is encoded by the CCL2 gene located on chromosome 17q11.217. In general, CCL2, particularly its role in mediating cell migration, is increasingly recognized as important in human pathological processes. It is important in the generation of adaptive immune responses and also

in the occurrence of different diseases such as rheumatoid arthritis. In this regard, CCL2 expression is caused by inflammatory stimuli and stimulates the recruitment of Th17 cells in particular locations resulting in the release of inflammatory cytokines and the development of pathological reactions¹⁸.

Chemokine CXCL12, also referred to as stromal cell-derived factor-1 (SDF-1) or pre-B-cell growth-stimulating factor (PBSF), is a member of the CXC chemokine family, distinguished by a single amino acid between its first two cysteine residues. This chemokine is pivotal in maintaining homeostatic control of leukocyte trafficking and plays a significant role in tissue repair. Additionally, CXCL12 is crucial for key physiological processes, including the homing of bone marrow-derived stem cells, cardiovascular system development, and neural guidance during embryonic growth¹⁹. This study was aimed to find that associated with vitamin D3 deficiency and some chemokines (CCL2, CXCL12) also study the effect of vitamin D3 deficiency on some coagulation factors (PT, FG and FVIII). In addition to studying some parameters such as (CRP and CBC).

Methodology

Study Design: This case-control study comprised 60 patients, and all of them were females diagnosed with severe vitamin D3 deficiency, with ages ranging from 18 to 65 years, during their attendance at medical laboratories in Baghdad. In addition, 30 healthy individuals of matching ages served as the control group. This study was conducted in the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, from January 2025 to September 2025.

Eligibility Criteria: This study had: a) exclusively female patients of varying heights and weights, b) their ages varied from 18 to 65 years, c) had vitamin D3 acute deficiency or a normal level, d) without ever suffering from blood clots, hypertension, diabetes, or any inflammation during the sample collection period. This research excluded patients: a) male, b) female with age below 18 and above 65, c) female with vitamin D3 level between 15 ng/ml and 30ng/ml, d) patients who suffered from hypertension, diabetes, blood clots, and any inflammation during the sample collection period.

Demographic and Clinical Cases: Ninety specimens of Iraqi women were chosen and gathered for participation in this investigation, in accordance with the conditions outlined in the inclusion criteria

paragraph above. Within the foundational questionnaire, participants provided information regarding their age, weight, height, health status (including hypertension, diabetes, and any current inflammations), family medical history, and if they were using vitamin D3 supplements, along with the dosage.

Collection of Samples: For each participant, five mL of venous blood was drawn using a five mL sterile syringe via venipuncture. The collected blood was then distributed into gel tubes, sodium citrate tubes and ethylenediaminetetraacetic acid (EDTA) tubes. Whole blood specimens obtained in EDTA tubes were utilized for CBC. Following the centrifugation of gel tubes at 3,000 rpm for 20 minutes, the separated serum was used to measure the required analysis tests represented by vitamin D3 level and immunological tests such as C-reactive protein (CRP). After that, it was transferred into an Eppendorf tube and stored at -20°C to measure immunological markers, including chemokines (CCL2 and CXCL12), using serum; also, centrifugation of sodium citrate tubes at 3,000 rpm for 20 minutes to isolate blood plasma for coagulation factor tests represented by PT, FG, and FVIII (Figure I).

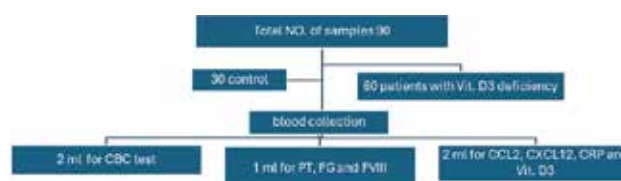


Figure I: Flow chart of study

Measurements of Biomarkers: The Human SDF-1 microsample ELISA kit (ELK biotechnology, ELK1183MS, China) and the Human MCP1 ELISA kit (ELK biotechnology, ELK5252, China) were employed to measure CCL2 and CXCL12 chemokine antibodies in serum samples from participants with or without vitamin D3 deficiency, also Human FG (Fibrinogen) ELISA Kit (ELK Biotechnology, ELK11104, China), Human F8 (Coagulation factor VIII) ELISA Kit (ELK Biotechnology, ELK10386, China) and Human PT (Prothrombin) ELISA Kit (Elabscience, E-EL-H1792, China). Ultimately, optical density (O.D.) readings were quantified at 450 nm utilizing a microtiter plate reader. AFIAS CRP (Boditech, China) detected antibodies in the buffer that bind to antigens in the sample, leading to the production of antigen-antibody complexes that migrate to the nitrocellulose matrix, where they are captured by

supplementary immobilized antibodies on the test strip. The VD-T II kit is an automated chemiluminescent immunoassay (CLIA) intended for the quantitative assessment of total 25-OH-vitamin D3 in serum specimens. The BC-30S Auto Hematology Analyzer, a computerized counting apparatus manufactured by Mindray (China), is employed for CBC evaluations, delivering results in less than one minute.

Statistical Analysis: The Statistical Package for the Social Sciences (SPSS) application (2019) was utilized to assess the impact of various groups on study parameters. The T-test and Least Significant Difference (LSD) were employed to compare means significantly. The Chi-Square test was employed to compare percentages significantly. Estimation of the correlation coefficient among various factors in patient groups within this study.

Ethical Clearance: Ethical approval was obtained from the relevant Institutional Ethics Committee before the study commenced. Written informed consent was obtained from all participants. All procedures were conducted in accordance with the Declaration of Helsinki.

Results

Demographics and Baseline Clinical Characteristics:

All participants identified as female and were of white ethnicity, reducing the potential confounding effect of skin pigmentation on vitamin D3 synthesis. The majority (100.0%) of the patient population reported living in the center of Baghdad. A total of 90 individuals were enrolled, including 60 patients and 30 healthy individuals as controls. The mean age of patients was (28.5±1.38) years, compared with (30.0± 1.75) years in controls, with no significant difference between groups (P = 0.5285) (Table 1).

Chemokine CCL2 and CXCL12 levels: A markedly significant increase (P ≤0.01) in CCL2 and CXCL12 was seen in the vitamin D3 deficiency group relative to the control group. The CCL2 level in the patients' group was (289.13 ± 4.30 pg/ml) and CXCL12 was (2.785 ± 0.08 ng/mL), in contrast to the control group, where the CCL2 level was (143.28 ± 7.24 pg/mL) and CXCL12 level was (0.676 ± 0.04 ng/mL) (Table 2, Figure II).

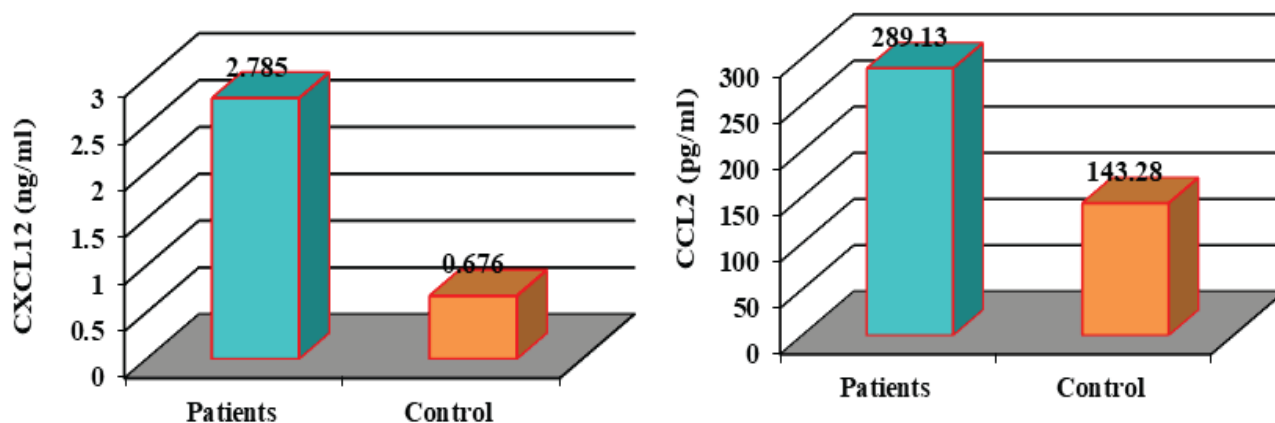
Coagulation Factors (PT, FG, FVIII) Levels: The results indicate that there were considerable

Table 1: Baseline Demographics and Baseline Clinical Characteristics of Patients and Controls

Variables	Group	
	Case	Control
Race/ ethnicity	60 White (100.0%)	30 White (100.0%)
Female	100.0%	100.0%
Mean Age±SD	28.50 ±1.38 years	29.97±1.75 years
Take D3 supplements	11/60(18.33%)	19/30(76.7%)
Age Group	Age Group	Age Group
Less Than 25 Years	27(45.0%)	12(40.0%)
25 to 35 Years	27(45.0%)	11(36.7%)
More Than 35 Years	6(10.0%)	7(23.3%)
Chronic Medical Conditions		
Hypertension	60 without 0%	30 without 0%
Diabetes	60 without 0%	30 without 0%
Current inflammation	52 without (86.7%)	27 without (90.0%)
Daily sun exposure		
< 15 min.	46 (76.7%)	9 (30.0%)
15 – 30 min.	10 (16.7%)	14 (46.67%)
> 30 min.	4 (6.67%)	7 (23.33 %)
Intake of vit. D rich food		
Rare	44 (73.3%)	5 (16.67%)
Occasional	16 (26.7%)	14 (46.67%)
Regular	0 (0%)	11 (36.67%)

Table 2: Level of Chemokine CCL2 and CXCL12 Concentration in Study Groups

Group	Means $\pm$ SE	
	CCL2 (pg/ml)	CXCL12 (ng/ml)
Case	289.13 $\pm$ 4.30	2.785 $\pm$ 0.08
Control	143.28 $\pm$ 7.24	0.676 $\pm$ 0.04
T-test	15.789 **	0.2428 **
P-value	0.0001	0.0001

**Figure II: Level of chemokines CCL12 and CXCL2 Concentrations in Studied Groups; (A) CXCL12. (B) CCL2**

differences in coagulation parameters of the studied groups. The levels of PT were lower in the patients than in the control group (74.88 ± 2.21 ng/ml and 191.23 ± 14.32 ng/ml, respectively; $p = 0.0001$). On the contrary, the level of factor VIII was much higher in patients than in controls (5.245 ± 0.16 IU/ml and 1.966 ± 0.10 IU/ml) respectively. Similarly, the levels of fibrinogen in patients were much greater than in the control group (2.436 ± 0.08 μ g/ml and 1.366 ± 0.09 μ g/ml) respectively (Table 3, Figure III).

Other Biomarkers (CRP and CBC) levels: The results revealed a significant increase ($P \leq 0.05$) in CRP values; CRP level in patients was (5.046 ± 0.83

mg/L), while in controls it was (2.90 ± 0.69 mg/L) (Table 4, Figure IV).

On the other hand, the CBC test (PLT, WBC, lymphocytes, RBC, HGB) revealed no significant and the value of (PLT, WBCs, Lymphocyte, RBCs and HGB) in patients were (275.66 ± 9.06 10^9 /L, 7.77 ± 0.27 10^9 /L, $31.19 \pm 1.27\%$, 4.54 ± 0.07 10^{12} /L, 12.27 ± 0.46 g/dl) respectively, while these values in controls group were (266.17 ± 11.50 , 7.57 ± 0.30 , 32.92 ± 0.62 , 4.51 ± 0.06 , 11.97 ± 0.17) respectively (Table 4, Figure IV).

Correlation between Biomarkers: Correlation analysis demonstrated distinct association patterns between serum vitamin D3 and inflammatory as

Table 3: Level of Coagulation Factors in Studied Groups

Group	Means $\pm$ SE		
	PT (ng/ml)	FVIII Congelation factor (IU/ml)	Fibrinogen (μ g/ml)
Patients	74.88 $\pm$ 2.21	5.245 $\pm$ 0.16	2.436 $\pm$ 0.08
Control	191.23 $\pm$ 14.32	1.966 $\pm$ 0.10	1.366 $\pm$ 0.09
P value	0.0001	0.0001	0.0001

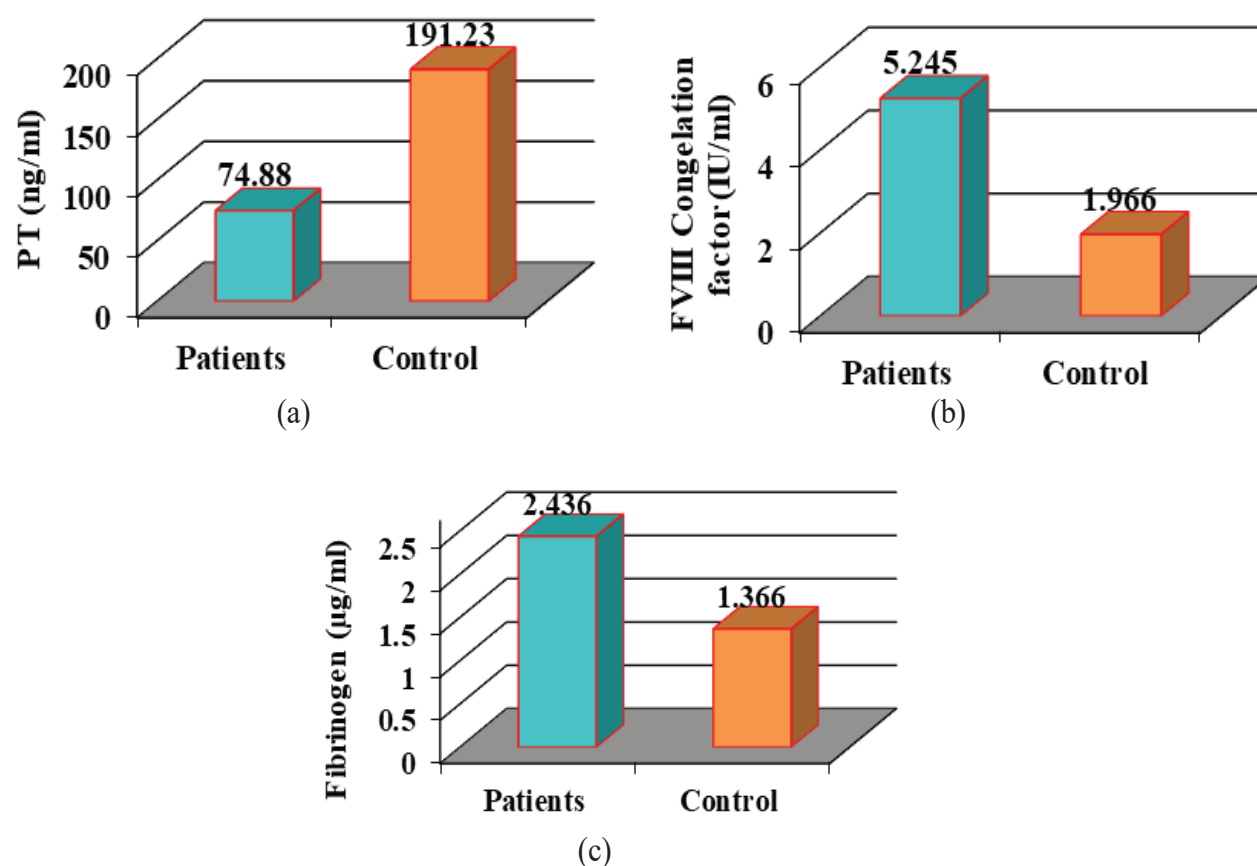


Figure III: The levels of coagulation factors PT, FG, FVIII concentration in studied groups; (A) PT (B) FVIII (C) FG

Table 4: Values of PLT, WBC, Lymphocytes, RBC, HGB and CRP in Studied Groups (Means $\pm$ SE)

Group	PLT ($10^9/L$)	WBC ($10^9/L$)	Lymphocyte (%)	RBC ($10^{12}/L$)	HGB (g/dl)	CRP (mg/L)
Case	275.66 $\pm$ 9.06	7.77 $\pm$ 0.27	31.19 $\pm$ 1.27	4.54 $\pm$ 0.07	12.27 $\pm$ 0.46	5.046 $\pm$ 0.83
Control	266.17 $\pm$ 11.50	7.57 $\pm$ 0.30	32.92 $\pm$ 0.62	4.51 $\pm$ 0.06	11.97 $\pm$ 0.17	2.90 $\pm$ 0.69
P-value	0.5331	0.6515	0.4175	0.7858	0.6487	0.0497

well as hematological parameters. In the patients group, vitamin D3 levels were significantly and inversely correlated with CXCL2 ($r = -0.22$, $p = 0.0497$), while no significant correlations were detected with PT, FVIII, FG, CXCL12, CRP, PLT, WBC, lymphocyte count, RBC, or hemoglobin ($p > 0.05$) conversely, in the control group, vitamin D3 exhibited a significant positive correlation with

prothrombin ($r = 0.26$, $p = 0.0417$), while significant negative correlation with WBC count ($r = -0.27$, $p = 0.0405$) and a significant positive correlation with hemoglobin levels ($r = 0.32$, $p = 0.00388$). All remaining parameters showed no statistically significant associations with vitamin D3 ($p > 0.05$) (Table 5).

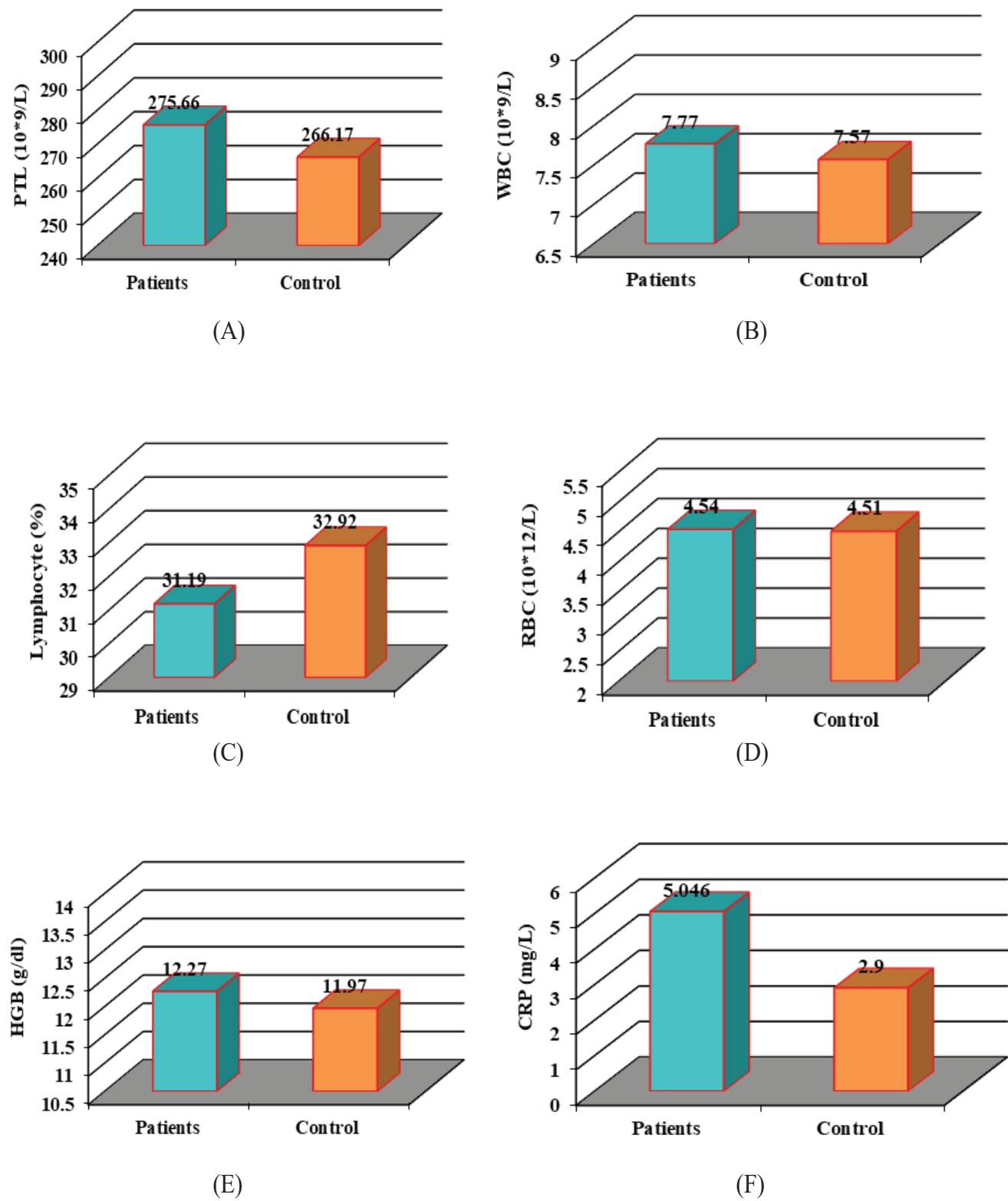


Figure IV: Level of Complete blood count (CBC) and C-reactive protein in studied groups; (A) PLT, (B) WBC, (C) Lymphocytes, (D) RBC, (E) HGB, and (F) CRP

Figure IV: Level of Complete blood count (CBC) and C-reactive protein in studied groups; (A) PLT, (B) WBC, (C) Lymphocytes, (D) RBC, (E) HGB, and (F) CRP

Parameters	Group			
	Case Group		Control Group	
PT	Correlation-r	P value	Correlation-r	P value
FVIII Congelation factor	0.13 NS	0.3074	0.26 *	0.0417
Fibrinogen	0.02 NS	0.8820	-0.11 NS	0.5488
CCL2	0.06 NS	0.6245	0.16 NS	0.4008
CXCL12	-0.22 *	0.0497	0.20 NS	0.2836
CRP	0.03 NS	0.8257	-0.01 NS	0.9485
PLT	0.09 NS	0.4835	-0.13 NS	0.4886
WBC	0.06 NS	0.6562	-0.07 NS	0.6874
Lymphocyte	-0.02 NS	0.8814	-0.27 *	0.0405
RBC	0.10 NS	0.4338	0.21 NS	0.2674
HGB	-0.07 NS	0.5904	0.09 NS	0.6374
	-0.06 NS	0.6540	0.32 *	0.0388

Discussion

The primary aim of the research was to examine the correlation between vitamin D3 levels and the key inflammatory, hematological and coagulation markers related to vitamin D3, especially the chemokines CCL2 and CXCL12 in a group of patients. This research revealed that the negative correlation between the levels of D3 and the chemokine CCL2 are statistically significant within the group of patients. The study outcomes are a strong argument in favor of the previously proven pleiotropic and anti-inflammatory action of vitamin D3 with reference to the pathology of the disease^{20,21}. CCL2 is a highly active chemokine that promotes the migration of monocytes, memory T cells, and dendritic cells to inflammatory sites, thereby serving a central role in the development and progression of multiple inflammatory and autoimmune conditions²². The negative correlation observed suggests that higher levels of vitamin D3 may attenuate the inflammatory response by downregulating CCL2 expression. This observation aligns with findings from both in vitro and in vivo studies, which indicate that the active form of vitamin D, 1,25-dihydroxyvitamin D, can directly suppress the production of multiple pro-inflammatory cytokines and chemokines, including CCL2, within immune cells and other tissue types^{23,24}. The clinical evidence indicates that this regulatory mechanism functions within the patient population, suggesting that D3 may mitigate excessive inflammatory signals transduced via CCL2. In contrast to the sick group, the connection between D3 and CCL2 in the healthy control group was not significant. The difference can indicate the state-dependent nature of the immunomodulatory effects of D3; its

immunosuppressive effect on inflammatory markers such as CCL2 is statistically significant and biologically relevant only under the condition of the activation of the inflammatory pathway, which is the case in the patient cohort.

Also, the study investigated the connection between D3 and CXCL12. No significant association was observed between vitamin D3 levels and CXCL12 in either the patient or control groups. Experimental studies have demonstrated that vitamin D3 deficiency can stimulate CXCL12 expression and enhance the activation of the CXCL12/C-X-C chemokine receptor type 4 (CXCR4) signaling axis in vivo. CXCR4 is a transmembrane cell surface receptor and one of the key regulators involved in multiple physiological and pathological processes. Its primary function is mediated through binding to its specific ligand, CXCL12, forming a signaling axis that plays a central role in cellular trafficking. This interaction critically regulates cell migration, homing, and retention within various tissues and organs. In addition, the CXCL12–CXCR4 axis contributes to immune cell recruitment, maintenance of tissue homeostasis, and modulation of inflammatory responses, highlighting its importance in both normal immune function and disease conditions²⁵.

This mechanism may account for the elevated CXCL12 levels observed in females with severe vitamin D3 deficiency, suggesting that insufficient vitamin D3 may promote chemokine-mediated inflammatory signaling pathways²⁶. The data indicate that vitamin D3 levels, within this specific clinical context, may not represent the primary or dominant regulator of CXCL12 expression. Furthermore, meta-analytic evidence has shown no consistent or systematic effect of vitamin D3 supplementation on

circulating levels of CRP²⁷, Mendelian randomization studies indicate the existence of a causal relationship between low levels of serum 25(OH)D and elevated levels of CRP, especially in the deficient range. In combination, these results are in line with an exaggerated inflammatory condition related to serious deficiency of vitamin D3²⁸. In the patient cohort, no significant correlation was found between vitamin D3 levels and the inflammatory marker CRP, indicating that the anti-inflammatory effects of vitamin D3 may be more specifically mediated through CCL2-related pathways rather than representing a broad suppression of all inflammatory mediators.

Moreover, observational studies have suggested an association between vitamin D3 deficiency and a prothrombotic state. A recent cohort investigation reported a dose-response relationship, with lower serum vitamin D3 concentrations linked to an increased risk of thrombotic events²⁹. Regularly, a systematic review revealed that the majority of observational studies associate the lack of vitamin D3 with changes in coagulation-related parameters³⁰. Nevertheless, the evidence is still heterogeneous because various studies have not been able to prove that vitamin D3 supplementation has a significant effect, and interventional data revealed that vitamin D3 supplementation does not always alter thrombin generation or fibrin clot profile³¹. The overall results of this research indicate that the effect of vitamin D3 status on coagulation might not be possible across populations but rather vary depending on the context³⁰. Patients exhibited reduced prothrombin levels compared with controls, alongside elevated FVIII activity and FG concentrations. This pattern reflects an altered hemostatic profile suggestive of a procoagulant shift associated with severe vitamin D3 deficiency in women³¹. Collectively, these findings indicate that vitamin D3 deficiency may be linked to modulation of key coagulation factors rather than isolated changes in individual parameters³³.

Interestingly, the control group had significant correlations of D3 and some hematological parameters, a negative correlation with WBC count, while a positive correlation with HGB levels. Serum vitamin D3 and WBC counts have shown a significant negative correlation, indicating that deficiency of vitamin D3 can be accompanied by increased immune or inflammatory response; vitamin D3 has immunomodulatory action on innate and adaptive immunity; therefore, reduced vitamin D3 levels may impair immune functioning and lead to a relative enhancement of the circulating leukocytes as an inflammation of low grade³⁴. Strong positive relationships between the levels of vitamin D3 and

hemoglobin concentration were stated, which showed that vitamin D3 deficiency can be connected with lower hemoglobin and increased vulnerability to anemia. Such a relationship is biologically possible since, throughout the body of adult observational studies, vitamin D3 status has been positively associated with reduced hemoglobin and increased prevalence of anemia, and thus interplay between vitamin D3 deficiency, inflammatory milieu, and erythropoietic efficiency³⁵. No significant correlation between serum levels of vitamin D3 and RBC count, platelet count, and lymphocyte percentage was found. This insignificance indicates that in the absence of evident hematologic or inflammatory pathology, vitamin D3 status may not be indicated by baseline hematologic cell counts and that effects of vitamin D3 on CBC indices may be inconsequential or smaller between populations³⁶.

In addition, recent findings suggest that correlations between vitamin D3 status and platelet indices are weak and may be indirect, mediated probably by low-grade inflammatory processes, not necessarily by direct action on thrombopoiesis; hence, null relationships between PLT (and corresponding proportions of immune cells) can be biologically plausible in cross-sectional studies³⁷. The last step was the inter-parameter correlation analysis of the patient group, which showed no significant CCL2 and CXCL12 inter-parameter relationships with CRP. This insignificance indicates independent regulation of these inflammatory mediators or that their concentrations are affected by other factors in the underlying disease that result in a non-linear relationship between them.

Conclusion

Severe vitamin D₃ deficiency was associated with increased CCL2 expression and elevated WBC counts, indicating enhanced chemokine-mediated inflammation. In contrast, CXCL12 and CRP levels remained unchanged, suggesting that vitamin D₃ deficiency selectively influences inflammatory pathways rather than causing generalized systemic inflammation. Hemoglobin levels were significantly reduced despite preserved RBC counts, reflecting impaired erythropoietic function without affecting red blood cell numbers. Patients also exhibited lower prothrombin levels together with increased factor VIII activity and fibrinogen, indicating a shift toward a procoagulant state. Platelet counts and lymphocyte percentages were not significantly altered, demonstrating that some hematological and immune parameters remain preserved. Overall, severe vitamin D₃ deficiency appears to selectively disrupt

inflammatory and coagulation homeostasis, highlighting its important role in immune regulation and hemostatic balance.

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Conflict of interest:

The authors would like to state that there is no conflict of interest in this work.

Financial Disclosure

External funding was not obtained for this research.

Authors' contributions

The entire work was done by Ghalib T. Al-Rashid under the supervision of dr. Prof. Zainab K. Hussien

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the ethical standards of the institutional research committee. Ethical approval was obtained from the relevant institutional ethics committee (approval code: CSEC/1025/0148 October 27, 2025). Written informed consent was obtained from all participants prior to inclusion in the study.

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