

Assessment of serum levels of vitamin D and tumor necrosis factor alpha in ulcerative colitis patients at Egyptian Military Hospitals: Cross-sectional observational study

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Abstract

Ulcerative colitis (UC) is a chronic inflammatory disorder confined to the colonic and rectal mucosa. The disease is marked by infiltration of immune cells, leading to excessive production of inflammatory mediators. A key pro-inflammatory cytokine that plays a vital role in the pathophysiology of ulcerative colitis is tumor necrosis factor-alpha (TNF- α). Low levels of vitamin D have been associated with several autoimmune disorders. This study aimed to assess vitamin D serum levels in UC patients as regard TNF- α serum levels and the Severity of UC (UCDAI). This cross-sectional observational research was carried out at Military healthcare facilities in Cairo, Egypt, over six months, from 1 July 2025 to 31 December 2025. Utilizing a convenience sample of 50 adults aged 15 to 70 years with a confirmed diagnosis of ulcerative colitis, newly diagnosed. They were divided into two groups according to disease severity (UCDAI or Mayo score. 26 patients in the mild group & 24 patients (moderate/ severe) group. Demographic data were collected from patients' interviews. Colonoscopy for ulcerative colitis disease activity index (UCDAI) score, CBC, ESR, CRP, and Fecal calprotectin (FC) were investigated at Egyptian Military hospitals. Serum levels of 25-hydroxyvitamin D (using CLIA) and TNF- α (using ELISA) were measured at central laboratories of the armed forces and blood bank. Correlation analysis showed Serum Vitamin D had a moderate negative correlation with UCDAI ($r = -0.466$, p -value= 0.001), and a strong negative correlation with serum TNF- α ($r = -0.669$, p -value <0.001). Serum TNF- α showed a moderate positive correlation with UCDAI ($r = 0.472$, p -value= 0.001). Fecal calprotectin had a strong positive correlation with UCDAI ($r = 0.704$, p -value <0.001) and a weak positive correlation with serum TNF- α ($r = 0.388$, p -value= 0.005). In conclusion, this study demonstrates that patients with moderate to severe ulcerative colitis had pronounced mucosal and systemic inflammation, reflected by markedly elevated fecal calprotectin and TNF- α levels, concomitant with significantly lower vitamin D concentrations compared with those in mild disease.

Keywords: Ulcerative colitis, Vitamin D, Tumor Necrosis Factor Alpha, Inflammatory bowel disease, Cytokines, Ulcerative colitis disease activity index.

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Introduction

Ulcerative colitis is an example of a chronic inflammatory bowel disease (IBD), which is defined by continuous mucosal layer inflammation extending from the rectum to the upper portions of the colon.¹ The Mayo score or activity index (UCDAI) is widely recognized as one of the measures of disease activity in placebo-controlled trials for ulcerative colitis (UC). Its full version consists of four components: rectal bleeding, stool frequency, physician evaluation, and endoscopic appearance.² Epidemiological studies have shown that ulcerative colitis is more common in higher latitudes with less exposure to sunlight and less natural vitamin D production than in lower latitudes.¹ Experimental data indicate that when vitamin D and Vitamin D Receptors (VDR) are deficient, IBD results from alterations in vitamin D levels and VDR signaling (The active biological form. Calcitriol interacts with the vitamin D receptor, which pairs with the retinoid X receptor. This complex connects to the vitamin D response element and moves into the cell nucleus, where it controls the expression of various genes related to calcium and phosphorus metabolism, along with the regulation of innate and adaptive immunity), as well as an increase in the production of certain pro-inflammatory cytokines, such as interferon- γ and tumor necrosis factor- α (TNF- α).^{3,4}

Conversely, according to the theory of reverse causality, vitamin D deficiency may result from ulcerative colitis. Because alterations in lifestyle, intestinal absorption, nutritional status, and food choices have been linked to gastrointestinal disorders. Reducing complications, promoting and maintaining recovery, and enhancing quality of life are the objectives of treatment for this illness.¹ Tumor necrosis factor α (TNF- α) is a cytokine protein recognized as a key player in the inflammatory and immune responses in mammals.⁵ During inflammatory responses, it can also be produced by a variety of cell types, including T

and B lymphocytes, mast cells, endothelial cells, neutrophils, cardiac cells, muscle cells, fibroblasts, osteoblasts, and natural killer (NK) cells.⁶ Clinical research has resulted in the identification of anti-TNF α monoclonal Antibodies, which have significantly transformed the treatment landscape for IBD.⁷ The need for anti-TNF α biologic therapies remains significant. The steep cost continues to restrict access to anti-TNF α biologic treatments in areas with limited resources.⁸ Growing evidence indicates that nutritional factors, particularly vitamin D status, play an important modulatory role in IBD activity, immune regulation, intestinal barrier integrity, and response to biologic therapy.⁹ It has been proposed that vitamin D may improve the likelihood of responding to anti-TNF- α therapy by inhibiting the expression of TNF- α -related genes in the gut. KH 1060, a vitamin D analog, was found to lower TNF- α levels in peripheral blood mononuclear cell (PBMC) cultures derived from IBD patients. This possible effect of vitamin D was supported by research conducted by Kuo *et al.*, which indicated that calcitriol inhibited TNF- α and Th1-related cytokines in lipopolysaccharide (LPS)-stimulated THP-1 cells and human primary monocytes. Amrollah, et al., demonstrated that vitamin D supplementation in patients with Mild-to-moderate ulcerative colitis reduces the inflammatory cytokines IL-12p70, IFN- γ , and TNF- α , but does not influence the circulating levels of IL-4 and IL-10.¹⁰ Vitamin D supplementation has been shown to potentiate the efficacy of TNF- α inhibitors and biologic therapies, particularly in inflammatory bowel disease and other TNF- α -driven conditions, suggesting a clinically relevant interaction with biologic drugs.¹¹ In Egypt, limited data are available regarding the interplay between serum vitamin D levels, TNF- α , and disease activity in UC patients. So, this study aimed to assess vitamin D serum levels in UC patients as regard TNF- α serum levels and severity of UC. The novelty of the present study

lies in the simultaneous evaluation of serum vitamin D and TNF- α in relation to graded UC disease activity in an Egyptian cohort, with assessment of their ability to distinguish between different severity categories.

Patients and Methods

Study design this cross-sectional observational study was carried out over six months, from 1 July 2025 to 31 December 2025. Study population consisted of patients with a confirmed diagnosis of ulcerative colitis, newly diagnosed at Egyptian military hospitals in Cairo, Egypt.

Subjects were grouped as follow: The study consisted of 50 adults with a confirmed diagnosis UC, grouped according to disease severity (UCDAI or Mayo score² into two groups: Mild group 26 cases & moderate/severe group 24 cases. Inclusion Criteria: Patients with a confirmed diagnosis of ulcerative colitis, newly diagnosed, aged between 15 and 70 years at the time of enrollment. Exclusion Criteria: Presence of chronic kidney or liver disease that may alter vitamin D metabolism, Diagnosis of other inflammatory bowel diseases (e.g., Crohn's disease, indeterminate colitis), Active acute or chronic infection at the time of blood sampling, Use of medications known to interfere with TNF- α or vitamin D levels (Biologics, anticonvulsants, Glucocorticoids, Antiviral therapy, Orlistat), Presence of conditions known to affect vitamin D or cytokine levels, such as autoimmune diseases, malignancies, short bowel syndrome, or intestinal failure, Pregnancy or lactation.

Methods

All patients were subjected to history taking, clinical examination, and laboratory investigations: Complete blood count (CBC), Erythrocyte sedimentation rate (ESR), C - reactive protein (CRP), and fecal calprotectin (FC) by stool analysis and colonoscopy were performed at Egyptian Military hospitals. Disease severity (UCDAI) or Mayo score was scored by four components (Rectal bleeding, stool frequency, endoscopic appearance, and Gastroenterologist global assessment). Each

component is scored from 0 to 3, resulting in an overall score that ranges from 0 to 12. Scores between 3 and 5 suggest mild disease activity, scores from 6 to 10 indicate moderate disease activity, and scores from 11 to 12 reflect severe disease activity.² Sample Processing and Storage for assessment of serum levels of 25-hydroxyvitamin D using Chemiluminescence Immunoassay (CLIA) kits (VITROS Eci/ECiQ/3600 Immunodiagnostic systems & VITROS 5600/XT 7600 Integrated systems) and TNF- α using Enzyme-Linked Immunosorbent Assay (ELISA) technique kits (Cat No: E0082Hu) BT LAB Bioassay Technology Laboratory). Venous blood samples were collected from all patients under strict aseptic conditions by trained phlebotomists and allowed to clot at room temperature for 20–30 minutes. Samples were centrifuged at 3000 rpm for 10 minutes to separate serum. Serum aliquots were transferred into labeled cryovials. Samples were stored at -20°C until laboratory analysis, in accordance with kit manufacturer recommendations.

Statistical Analysis

The collected data were revised, coded, tabulated, and introduced to a PC using the Statistical Package for Social Science (SPSS 27). Data were presented, and suitable analysis was done according to the type of data obtained for each parameter. Descriptive statistics: Mean, Standard deviation ($\pm$ SD), and range for parametric numerical data, while Median and Interquartile range (IQR) for non-parametric numerical data. Frequency and percentage of non-numerical data. Analytical statistics: Mann-Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. Correlation analysis (using Spearman's rho method) to assess the strength of association between two quantitative variables. The correlation coefficient, denoted symbolically as "r" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables. The ROC Curve (Receiver Operating Characteristic) provides a useful way to evaluate the Sensitivity and specificity for quantitative Diagnostic

measures that categorize cases into one of two groups.

Results

This study was conducted on 50 ulcerative colitis patients to assess vitamin D serum levels in ulcerative colitis patients as regard TNF- α serum levels and the Severity of UC in these patients.

The mean age of the studied patients was 31.64 ± 12.64 years (range 14–66). Females

constituted the majority with 38 (76%) compared to 12 males (24%). Only 1 patient (2%) was a smoker, while 49 (98%) were non-smokers. Regarding sun exposure, 23 patients (46%) reported exposure compared to 27 (54%) who did not. Dietary vitamin D intake was absent in 41 (82%) and present in 9 (18%). Physical activity was practiced by 41 (82%) of the group, while 9 (18%) reported no activity (Table 1).

Table 1. Demographic data and risk factors for the study group.

		N (%)
Age (Years) Mean $\pm$ SD		31.64 $\pm$ 12.64
Range		(14 - 66)
Gender	Male	12 (24%)
	Female	38 (76%)
Smoking	No	49 (98%)
	Yes	1 (2%)
Sun exposure	No	27 (54%)
	Yes	23 (46%)
Diet Vitamin D	No	41 (82%)
	Yes	9 (18%)
Physical activity	No	9 (18%)
	Yes	41 (82%)

The ulcerative colitis disease activity index (UCDAI) had a median (IQR) 6 (4–9) and a range of 3–12. When interpreted, 26 (52%) had mild activity, 20 (40%) moderate activity, and 4 (8%)

severe activity. When grouped, 26 patients (52%) fell into the mild category, while 24 (48%) were classified as moderate/severe (Table 2).

Table 2. Ulcerative colitis disease activity index for the study group.

		N (%)
UCDAI Median (IQR)		6 (4 - 9)
Range		(3 - 12)
UCDAI interpretation	Mild	26 (52%)
	Moderate	20 (40%)
	Severe	4 (8%)
UCDAI severity groups	Mild	26 (52%)
	Moderate/Severe	24 (48%)

Laboratory investigations revealed a mean hemoglobin (Hgb) of 12.14 ± 1.3 g/dL (range: 7.7–14.3). The total leukocyte count (TLC) was $6.01 \pm 1.82 \times 10^3/\mu\text{L}$ (range 1.7–10.1), while platelet count (PLT) averaged $295.62 \pm 67.61 \times 10^3/\mu\text{L}$ (range 140–644). Erythrocyte sedimentation Rate (ESR) had a median of 24.5 (18–30) range: 9–46 mm/hr, and C- reactive

protein (CRP) was 12.5 (9–16) range: 3.5–40.6 mg/L. Fecal calprotectin (FC) was elevated with a median of 318 (148–470) range: 35–1857 $\mu\text{g/g}$. Serum vitamin D (S. Vit D) had a mean of 17.65 (13.4–25) range: 6–44.5 ng/ml, and serum TNF- α (S.TNF- α) had a median of 240 (180–350) range: 9–950 pg/ml (Table 3).

Table 3. Laboratory investigations for the study group.

	Mean $\pm$ SD Median (IQR)	Range
Hgb (g/dL)	12.14 ± 1.3	(7.7 - 14.3)
TLC $\times 10^3/\mu\text{L}$	6.01 ± 1.82	(1.7 - 10.1)
PLT $\times 10^3/\mu\text{L}$	295.62 ± 67.61	(140 - 644)
ESR (mm/hr)	24.5 (18 - 30)	(9 - 46)
CRP (mg/L)	12.5 (9 - 16)	(3.5 - 40.6)
Fecal Calprotectin (mcg/g)	318 (148 - 470)	(35 - 1857)
S. Vit D (ng/ml)	17.65 (13.4 - 25)	(6 - 44.5)
S. TNF- α (pg/ml)	240 (180 - 350)	(9 - 950)

Biomarker comparison between UCDAI severity groups revealed that fecal calprotectin was significantly higher in moderate/severe cases, with a median of 447 (342–763) $\mu\text{g/g}$ compared to 173 (110–301) $\mu\text{g/g}$ in mild cases (p-value <0.001). Serum vitamin D was lower in moderate/severe cases, median 14.15 (11.75–

19.55) ng/ml versus 20.45 (15.9–25.2) ng/ml in mild cases (p-value= 0.010). Serum TNF- α was higher in moderate/severe cases with a median of 335 (220–450) pg/ml compared to 200 (165–270) pg/ml in mild cases (p-value= 0.009) (Table 4), Figures 1, 2,3.

Table 4. Association between F. Calprotectin, S. Vit D & S. TNF- α and UCDAI severity groups.

	UCDAI severity groups		p-value
	Mild Median (IQR) Range	Moderate/Severe Median (IQR) Range	
Fecal Calprotectin (mcg/g)	173 (110 - 301) (35 - 473)	447 (342 - 763) (150 - 1857)	<0.001
S. Vit D (ng/ml)	20.45 (15.9 - 25.2) (8.1 - 44.5)	14.15 (11.75 - 19.55) (6 - 33.16)	0.010
S. TNF- α (pg/ml)	200 (165 - 270) (9 - 925)	335 (220 - 450) (15 - 950)	0.009

$p \leq 0.05$ is significant.

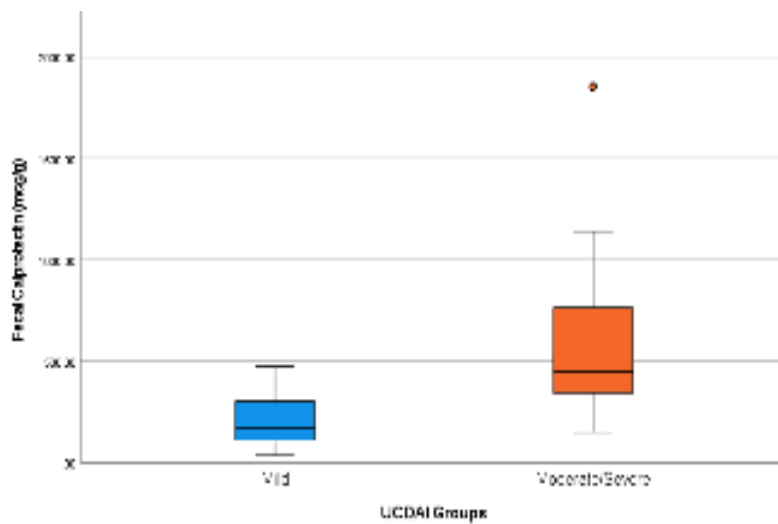


Figure 1. Shows difference in F. Calprotectin level between two severity groups.

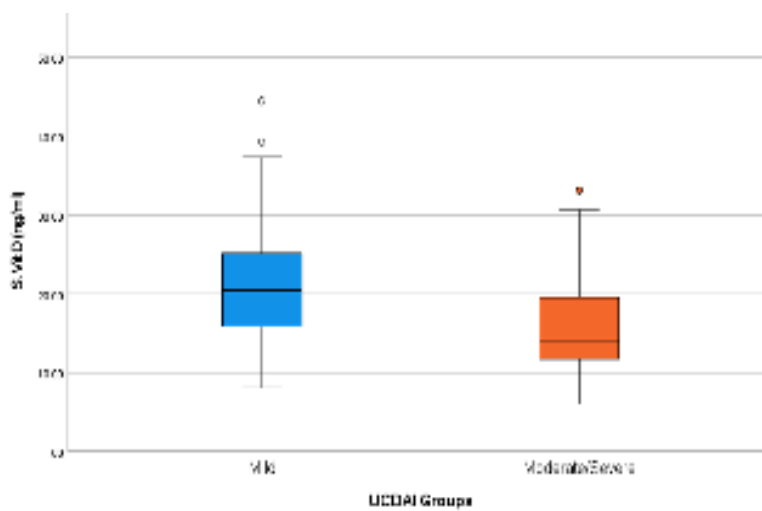


Figure 2. Shows difference in S. Vit D level between two severity groups.

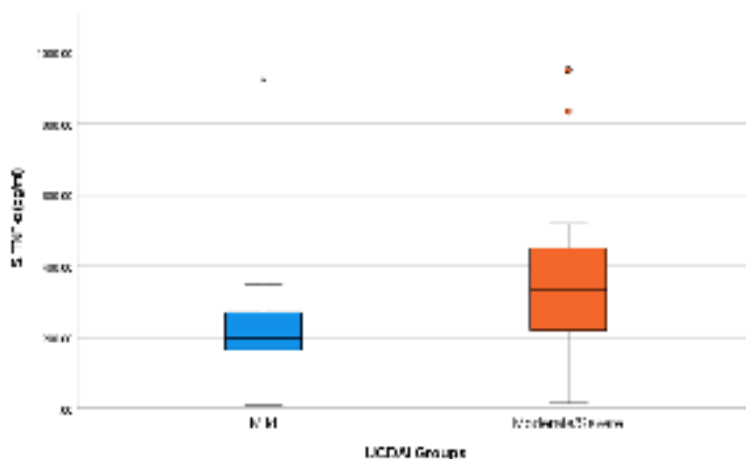


Figure 3. Shows difference in S. TNF-α level between two severity groups.

Correlation analysis showed fecal calprotectin had a strong positive correlation with UCDAI ($r = 0.704$, p -value <0.001), moderate correlations with ESR ($r = 0.573$, p -value <0.001) and CRP ($r = 0.529$, p -value <0.001), and a weak positive correlation with serum TNF- α ($r = 0.388$, p -value = 0.005). Serum vitamin D had a moderate negative correlation with UCDAI ($r = -0.466$, p -

value = 0.001), weak negative correlations with ESR ($r = -0.289$, p -value = 0.042) and CRP ($r = -0.352$, p -value = 0.012), and a strong negative correlation with serum TNF- α ($r = -0.669$, p -value <0.001). Serum TNF- α showed a moderate positive correlation with UCDAI ($r = 0.472$, p -value = 0.001) and with CRP ($r = 0.364$, p -value = 0.011) (Table 5).

Table 5. Correlation between F. Calprotectin, S. Vit D & S. TNF- α and different parameters.

		UCDAI	ESR (mm/hr)	CRP (mg/L)	S. Vit D	S. TNF- α
Fecal Calprotectin (mcg/g)	r	0.704	0.573	0.529	-0.197	0.388
	p -value	<0.001	<0.001	<0.001	NS	0.005
S. Vit D	r	-0.466	-0.289	-0.352	—	-0.669
	p -value	0.001	0.042	0.012	—	<0.001
S. TNF- α	r	0.472	0.270	0.364	—	—
	p -value	0.001	NS	0.011	—	—

r : Spearman's rho. $p > 0.05$ is not significant (NS).

ROC curve analysis demonstrated that fecal calprotectin had excellent diagnostic performance in predicting severe UC with an AUC of 0.899 (95% CI 0.818–0.98, p -value <0.001) at a cutoff >301 $\mu\text{g/g}$, yielding sensitivity 87.5%, specificity 76.92%, PPV 77.8% and NPV 87.0%. Serum vitamin D showed fair performance with an AUC of 0.712 (95% CI

0.561–0.862, p -value = 0.006) at a cutoff ≤ 14.2 ng/ml, sensitivity 58.33%, specificity 88.46%, PPV 82.4% and NPV 69.7%. Serum TNF- α had similar fair accuracy with an AUC of 0.716 (95% CI 0.567–0.864, p -value = 0.005) at a cutoff >290 pg/ml, sensitivity 54.17%, specificity 88.46%, PPV 81.2% and NPV 67.6% (Table 6).

Table 6. Diagnostic performance of F. Calprotectin, S. Vit D & S. TNF- α to predict severe cases of UC from non-severe cases.

	AUC	95% CI	p -value	Cut-off value	Sensitivity	Specificity	+PV	-PV
Fecal Calprotectin (mcg/g)	0.899	0.818 - 0.98	<0.001	>301	87.50%	76.92%	77.8%	87.0%
S. Vit D (ng/ml)	0.712	0.561 - 0.862	0.006	≤ 14.2	58.33%	88.46%	82.4%	69.7%
S. TNF- α (pg/ml)	0.716	0.567 - 0.864	0.005	>290	54.17%	88.46%	81.2%	67.6%

$p \leq 0.05$ is significant.

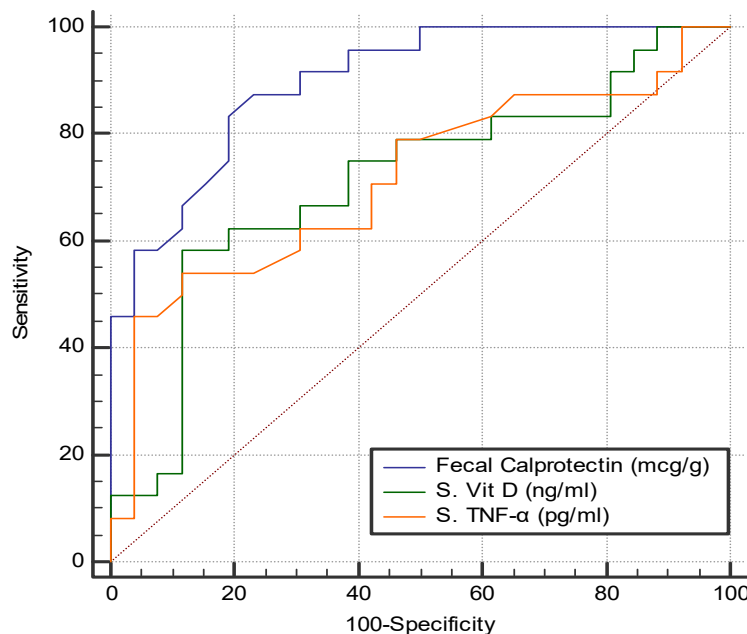


Figure 4. Shows ROC curve analysis for F. Calprotectin, S. Vit D & S. TNF- α to predict severe cases of UC.

Discussion

Inflammatory bowel diseases (IBD), comprising ulcerative colitis, are chronic relapsing inflammatory disorders of the gastrointestinal tract.¹²

Ulcerative colitis (UC) is a chronic inflammatory disorder confined to the colonic and rectal mucosa, characterized by recurrent phases of remission and exacerbation. The disease is marked by infiltration of immune cells, including macrophages, neutrophils, and lymphocytes, into the intestinal mucosa, leading to excessive production of inflammatory mediators.¹³ Dysregulation of T-lymphocyte function and altered cytokine signaling contribute to the persistence of mucosal inflammation. Among these mediators, Tumor necrosis factor- α (TNF- α) plays a pivotal role in the pathogenesis of ulcerative colitis, promoting leukocyte recruitment, disrupting epithelial barrier integrity, and sustaining inflammatory cascades within the gut. The central importance of TNF- α is supported by the proven efficacy of anti-TNF biological therapies in reducing disease activity and improving clinical outcomes, highlighting TNF- α as a key driver of intestinal inflammation in UC.¹⁴

Vitamin D, traditionally recognized for its role in bone metabolism, has emerged as an important immunomodulatory factor in UC. Through activation of the vitamin D receptor expressed on immune cells, vitamin D regulates antigen presentation, T-cell differentiation, and cytokine production, thereby maintaining intestinal immune homeostasis.¹⁵ The active form, 1,25-dihydroxyvitamin D₃, suppresses pro-inflammatory Th1 and Th17 responses and downregulates key cytokines implicated in UC pathogenesis, including TNF- α , while promoting anti-inflammatory Th2 cytokines and regulatory T-cell function. Experimental and genetic studies demonstrate that vitamin D deficiency or impaired vitamin D receptor (VDR) signaling exacerbates intestinal inflammation and increases susceptibility to UC. However, despite strong biological plausibility, clinical studies in humans have reported inconsistent associations between vitamin D levels, disease activity, and response to therapy, leaving the precise role of vitamin D deficiency in UC pathogenesis unresolved.¹⁶

Given the high burden of ulcerative colitis and the potential modifiable role of vitamin D in immune regulation, further investigation is warranted, particularly in regions with a high

prevalence of hypovitaminosis D.¹⁵ In Egypt, limited data are available regarding the interplay between serum vitamin D levels, TNF- α , and disease activity in UC patients.

Therefore, the present cross-sectional observational study was designed to assess serum levels of vitamin D and TNF- α in patients with UC attending Egyptian military hospitals, and to evaluate their associations with clinical and laboratory markers of disease activity.

In this study of 50 newly diagnosed patients with ulcerative colitis, the mean age was 31.64 $\pm$ 12.64 years, with a clear female predominance (76%). Most patients were non-smokers (98%). A high proportion of patients reported modifiable risk factors for vitamin D deficiency, including inadequate sun exposure (54%) and lack of vitamin D dietary supplement intake (82%), despite the majority reporting regular physical activity (82%).

The predominance of female patients in this cohort aligns with observations from several regional studies suggesting that women may have higher susceptibility to vitamin D deficiency, particularly in the context of IBD, due to differences in sun exposure habits and nutritional intake. The high prevalence of physical activity (82%) in our cohort contrasts somewhat with prior studies, which often report lower activity levels in IBD patients, potentially reflecting cultural or regional differences in lifestyle factors.¹⁷

Regarding the history of present illness, all patients in this study were newly diagnosed; they were not receiving biologic therapy or any treatment at the time of assessment. These characteristics provide an opportunity to evaluate the intrinsic relationship between vitamin D status, inflammatory activity (UCDAI), and TNF- α .

These observations are consistent with findings from Koureta, et al.¹⁸ who reported that the majority of UC patients in their cohort were early in the disease course and were not on vitamin D-modifying or biologic treatments, allowing for assessment of baseline serum vitamin D levels in relation to inflammatory markers.

Analysis of the association between inflammatory markers and UCDAI severity in the

present study demonstrated a clear and statistically significant relationship between disease activity and inflammatory burden. Patients with moderate to severe UC exhibited a marked elevation in fecal calprotectin (FC), with a median level of 447 μ g/g (IQR: 342–763) compared to 173 μ g/g (IQR: 110–301) in patients with mild disease ($p < 0.001$). This nearly 2.6-fold increase underscores the strong association between FC and mucosal inflammation as disease severity progresses.

These findings are consistent with those reported by Shahverdi, et al.,¹⁹ who demonstrated significantly higher FC levels in patients with active UC compared to those in mild or remission, confirming its reliability as a non-invasive marker of intestinal inflammation. Similarly, Koureta, et al.¹⁸ reported that FC levels rose proportionally with UCDAI scores, reinforcing its utility in stratifying disease severity. Large observational cohorts further support the strong correlation between elevated FC and endoscopic disease activity in UC patients.

In parallel, serum vitamin D levels were significantly lower in patients with moderate to severe disease, with a median of 14.15 ng/mL (IQR: 11.75–19.55) compared to 20.45 ng/mL (IQR: 15.9–25.2) in the mild group ($p = 0.010$). Importantly, vitamin D levels in the moderate/severe group fell within the range of deficiency (<20 ng/mL), indicating a clinically relevant inverse association between vitamin D status and UC severity (UCDAI).

These results align with those of Veit, et al.²⁰ and Fatahi, et al.,²¹ who reported that vitamin D deficiency was significantly more prevalent among patients with active UC and was associated with higher inflammatory markers and worse clinical outcomes. Additionally, Veit, et al.²⁰ demonstrated that lower serum vitamin D levels were associated with increased disease activity indices and inflammatory markers, supporting the hypothesis that vitamin D plays an immunomodulatory role in UC.

Furthermore, serum TNF- α levels were significantly elevated in patients with moderate to severe disease, with a median concentration of 335 pg/mL (IQR: 220–450) compared to 200 pg/mL (IQR: 165–270) in patients with mild

disease ($p = 0.009$). This represents an approximately 1.7-fold increase, highlighting the central role of TNF- α in mediating active intestinal inflammation.

The same findings have been reported by Vernia, et al.²² and Valvano, et al.⁹ who observed significantly higher TNF- α levels in patients with active UC compared to those in mild or remission, emphasizing its contribution to disease progression and severity. Shahverdi, et al.¹⁹ similarly documented elevated TNF- α concentrations in active UC, with positive correlations to disease activity indices and inflammatory markers.

Notably, serum vitamin D levels showed significant inverse correlations with UCDAI ($r = -0.466$, $p = 0.001$), ESR ($r = -0.289$, $p = 0.042$), CRP ($r = -0.352$, $p = 0.012$), and TNF- α ($r = -0.669$, $p < 0.001$). The particularly strong negative association with TNF- α may highlight a potential immunomodulatory role of vitamin D in suppressing pro-inflammatory cytokine production. These findings are consistent with Gubatan, et al.,²³ who demonstrated that lower vitamin D levels were associated with increased TNF- α expression and worse clinical outcomes in UC patients.

Furthermore, TNF- α itself was positively correlated with UCDAI ($r = 0.472$, $p = 0.001$) and CRP ($r = 0.364$, $p = 0.011$), confirming its role as a key mediator of both clinical severity and systemic inflammation. Similar correlations were described by Valvano, et al.,⁹ who reported significantly higher TNF- α levels in patients with active UC compared to those in mild or remission, alongside parallel increases in CRP and disease activity indices (UCDAI).

The ROC analysis showed that FC had the highest discriminatory performance among the three investigated parameters, with an AUC of 0.899. Vitamin D and TNF- α showed lower AUC values of 0.712 and 0.716, respectively. These findings suggest that FC had better discrimination between the predefined disease-severity groups than either serum vitamin D or TNF- α in this sample. Although the identified vitamin D and TNF- α cut-off values demonstrated relatively high specificity, their sensitivity was limited. Therefore, these cut-off values should not be considered diagnostic

thresholds or validated clinical decision points. Rather, they should be regarded as exploratory findings that require confirmation in larger independent cohorts.

The findings of this study may have practical implications. Assessment of vitamin D status may be useful in patients with UC, particularly in those with active or moderate-to-severe disease, because vitamin D insufficiency was associated with greater inflammatory activity in the present cohort. Vitamin D assessment may therefore be considered as part of the overall nutritional and clinical evaluation of patients with UC. However, the present study does not establish that vitamin D supplementation can reduce TNF- α levels or improve UC activity. Evidence from interventional studies is required before any therapeutic effect can be inferred. Previous work has suggested that vitamin D supplementation may influence inflammatory cytokines and response to anti-TNF therapy.^{10,11}

Limitations

1. Sample Size: The study included 50 patients, which limits the generalizability of the findings to the broader UC population.
2. Cross-Sectional Design: The observational, cross-sectional nature of the study prevents establishing causality between vitamin D deficiency, TNF- α levels, and disease severity.
3. Single-Center Study: Conducted in Egyptian military hospitals, the results may not fully represent UC patients in other geographic regions or healthcare settings.
4. Limited Assessment of Confounders: Factors influencing vitamin D status, such as dietary habits, sunlight exposure, seasonal variation, and comorbidities, were only partially controlled.
5. Absence of Longitudinal Follow-Up: Changes in vitamin D or TNF- α levels over time in relation to disease progression or treatment response were not assessed.

Conclusion

This study demonstrates that patients with moderate to severe ulcerative colitis had pronounced mucosal and systemic inflammation, reflected by markedly elevated

fecal calprotectin and TNF- α levels, concomitant with significantly lower vitamin D concentrations compared with those in mild disease.

Recommendations

1. Assessment of Vitamin D: Serum vitamin D levels should be monitored in UC patients as part of routine evaluation, particularly in those with moderate to severe disease activity.
2. Vitamin D Supplementation: Consider supplementation in deficient patients as a potential adjunctive strategy to modulate immune response and reduce TNF- α -mediated inflammation.
3. Longitudinal Studies: Future research should adopt prospective designs to evaluate causal relationships between vitamin D, TNF- α , and disease activity over time.
4. Multi-Center Research: Larger, multi-center studies are recommended to validate these findings and improve generalizability across different populations.
5. Integration with Therapy Monitoring: Assess the combined impact of vitamin D status and biologic therapies, such as anti-TNF agents, on disease outcomes to optimize personalized treatment strategies.

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Author Contributions

All authors certify that they participated in this work, including participation in study design, collecting data, analysis and interpretation of data, practical part, and writing the manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Ethical Committee Authority of the Military Medical Academy. The study was conducted in accordance with the Declaration of Helsinki.

Informed consent

Before being enrolled in the study, all participants provided written informed consent..

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