BMC Gastroenterology

RESEARCH

Open Access

# The role of vitamin D in treated and refractory ulcerative colitis patients: a case-control study

Amjad Ahmadi[1,2], Leili Shokoohizadeh[1,2], Farshad Sheikhesmaili[3], Bahram Nikkhoo[3], Asadollah Mohammadi[4], Mohammadali Khan Mirzaei[5,6], Mohammad Yousef Alikhani[1,2*] and Rasoul Yousefimashouf[2*]

## Abstract

**Background** Ulcerative colitis is a form of chronic inflammatory bowel disease (IBD) marked by ongoing inflammation of the mucosal lining that extends from the rectum to the upper part of the colon. Vitamin D regulates immune responses in several autoimmune and inflammatory diseases, including ulcerative colitis. Therefore, the study aims to investigate the role of vitamin D in the pathogenesis and treatment of ulcerative colitis.

**Methods** This case-control study included 94 participants who were divided into four groups. Group 1: people with ulcerative colitis who responded to treatment ($n=24$). Group 2: family members of patients who responded to treatment and did not have the disease ($n=24$). Group 3: People with ulcerative colitis who are resistant to treatment ($n=23$). Group 4: family members of treatment-resistant patients who does not have the disease ($n=23$). Groups 1 and 3 were considered as patient groups ($n=47$) and groups 2 and 4 as control groups ($n=47$). Blood samples were taken and analyzed for complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum vitamin D levels.

**Results** The mean age of treatment-responsive patients (group 1) was $45.88 \pm 18.51$ years, while treatment-resistant patients (group 3) averaged $41.30 \pm 13.01$ ($P=0.33$) years. Serum Vitamin D levels were $24.96 \pm 9.66$ ng/mL in group 1 and $27.70 \pm 12.28$ ng/mL in group 3, showing no significant correlation with ulcerative colitis ($P=0.41$). All groups had a BMI within the normal range, and mean CRP levels varied significantly across groups. Hemoglobin was significantly lower in group 3 compared to group 1 ($P=0.029$), but ESR results showed no significant relationship with ulcerative colitis. Vitamin D levels were highest in patients with lower BMI, and no significant relationships were found between Vitamin D and other risk factors, although extensive colitis was associated with higher Vitamin D levels compared to distal colitis.

**Conclusion** In this study, there was no significant association between ulcerative colitis and serum levels of vitamin D. However, the small number of patients may limit the conclusions that can be drawn regarding the role of vitamin D in the treatment of ulcerative colitis. Future studies should aim for larger cohorts to provide more definitive insights into this important issue.

**Keywords** Ulcerative colitis, Vitamin D, ESR, CRP

*Correspondence:
Mohammad Yousef Alikhani
alikhani43@yahoo.com; alikhani@umsha.ac.ir
Rasoul Yousefimashouf
yousefimash@yahoo.com

Full list of author information is available at the end of the article

## Introduction

Ulcerative colitis is a form of chronic inflammatory bowel disease (IBD) marked by ongoing inflammation of the mucosal layer, which ranges from the rectum to the upper parts of the colon [1, 2]. Common symptoms of this disease include diarrhea, bloody stools, loss of appetite, abdominal pain, nausea, vomiting, and weight loss. In some cases, extraintestinal manifestations may also be present [3, 4]. The diagnosis of this disease is primarily based on endoscopy, which usually reveals evidence of ongoing colonic inflammation. Biopsy specimens confirm symptoms of chronic colitis [3, 4]. The disease has two peak ages of onset: between 15 and 30 years old, and between 60 and 80 years old. The male-to-female ratio for this disease is 1:1 [5, 6].

Vitamin D is a fat-soluble secosteroid that can be synthesized from 7-dehydrocholesterol when the skin is exposed to ultraviolet light, or it can be obtained through dietary sources [7, 8]. Absorption of vitamin D depends on the ability of the intestine to absorb fat [7, 8]. Certain types of digestive diseases can cause malabsorption of fat and as a result fat-soluble vitamins [7, 8]. The classical functions of vitamin D are maintaining normal blood levels of calcium and phosphorous via intestinal absorption or renal reabsorption and, thus, bone health [7, 8]. Vitamin D is essential for the effective operation of both the innate and adaptive immune systems [9]. Additionally, It has anti-inflammatory and immunomodulatory properties [9]. The availability of vitamin D is critical for the integrity of intestinal epithelium, improving barrier function, regulating mucosal immunity, and T-cell growth and function [10–12]. These functions of vitamin D are beneficial for suppressing unwanted immune reactions to prevent autoimmune and inflammatory diseases [13–15]. Low levels of vitamin D have been associated with various health issues, including infectious diseases, cancer, diabetes, depressive disorders, osteoporosis, autoimmune conditions, and inflammatory diseases like Crohn's disease and ulcerative colitis [13–15].

Epidemiological studies have shown a greater incidence of ulcerative colitis in higher latitudes with less sunlight exposure and less natural synthesis of vitamin D compared to lower latitudes [16, 17]. These data led to the hypothesis that low levels of vitamin D increase the risk of ulcerative colitis. On the other hand, based on reverse causality, ulcerative colitis could lead to lower vitamin D levels [16, 17]. Because in gastrointestinal diseases changes in dietary choices, intestinal absorption, nutritional status, and lifestyle were observed [16, 17]. Treatment goals for this disease are to induce and maintain recovery, reduce complications, and improve quality of life [17, 18]. Studies using animal models have shown that vitamin D supplementation can effectively treat ulcerative colitis [17, 18]. Also, several studies involving human IBD patients including both randomized clinical trials (RCTs) and non-RCTs have been published in this issue and showed beneficial effects of vitamin D [19–21]. Moreover, few meta-analyses have been published [22, 23]. Due to very low-certainty evidence or missing data, these studies could not make any clear recommendations. However, it is unclear whether vitamin D deficiency in individuals with ulcerative colitis is a nutritional cause or a consequence of the disease. Also, vitamin D deficiency in ulcerative colitis patients compared to healthy subjects or patients with high vitamin D levels may be inversely related to non-response to biological therapies. This study aims to investigate the relationship between serum vitamin D levels in treated and refractory ulcerative colitis patients.

## Materials and methods

### Ethical approval

The study was approved by the Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran (Ethic code No: IR.UMSHA.REC.1401.787). From all subjects written informed consent was obtained before participation in the study.

### Study population

Considering the power of 80% and the type I error of 0.05 and taking into account the effect size of 0.80, the sample size calculated was equal to 21 people for each group, which was determined more than that in our study. Also, considering that three control groups were considered, it is expected that the effect size will be more accurate and the type II error will be reduced.

All selected patients with ulcerative colitis were evaluated clinically and underwent further examinations using biochemical tests, colonoscopy, and biopsy. Biopsy samples were taken from the affected area in the large intestine with medical indications. Each biopsy sample was used for hematoxylin and eosin (H&E) staining and histopathological examination in the pathology laboratory. For ulcerative colitis patients treatment, three steps were performed as follows: The first stage was 5-aminosalicylic acid treatment. The second stage was glucocorticoids and azathioprine treatment and the third stage was anti-TNF-α. This case-control study included 94 participants who were divided into four groups.

Group 1: People with ulcerative colitis who responded to any of the three stages of treatment (treatment-responded patients; $n=24$). Group 2: The family members of patients who responded to treatment and did not develop the disease ($n=24$). Group 3: People with ulcerative colitis who did not respond to all three stages of treatment (treatment-resistant patients; $n=23$). Group 4: The family members of treatment-resistant patients who did not develop the disease ($n=23$). Groups 1 and 3 were

considered as patient groups ($n=47$) and groups 2 and 4 as control groups ($n=47$). The control groups live with the patient in the same situation, environment, and family and have the same age and gender. Inclusion criteria for patients in the study were ulcerative colitis patients who responded to at least one of the treatment steps (group 1) and patients who did not respond to any of the treatment steps (group 3). Exclusion criteria from the study were: people with diabetes, people with rheumatism, people with irritable bowel syndrome, people with immune system deficiencies, taking antibiotics within 6 months before sampling, and patients with renal failure.

#### Peripheral blood collection and serum separation

After obtaining informed consent, 5 mL of peripheral blood was collected from each participant and drawn in vacutainer vials. Tests performed in groups 1 and 3 included complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum vitamin D levels. In the second and fourth groups, C-reactive protein (CRP) and serum vitamin D levels were measured.

#### CBC and ESR measurement

The whole blood samples taken from the people immediately were taken to the medical diagnostic laboratory and the ESR (Automatic ESR analyzer PKL PPC 840 from the Italy company) and CBC (Mindray cell counter from the Indian company) were measured, but the serum samples for measuring vitamin D levels and CRP was kept at -20 °C until all the samples were collected.

#### Vitamin D and CRP measurement

The enzyme-linked immunosorbent assay (ELISA) technique was employed to assess serum vitamin D levels. A commercial kit from Pishtaz Teb Zaman Diagnostics (Tehran, Iran), was utilized for measuring both vitamin D and C-reactive protein (CRP). The serum levels of CRP were categorized as negative (≤6) or positive (>6), while vitamin D levels were classified as follows: deficient (<20 ng/mL), insufficient (20–30 ng/mL), sufficient (30–100 ng/mL), and potentially toxic (>100 ng/mL) [24].

#### Statistical analysis

Statistical analysis was performed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). The normality assumption was tested using the Shapiro-Wilk test. If the normality assumption was met, the assumption of equal variance in the two groups was tested. The t-test was used to compare the treatment and treatment-resistant groups, the treatment groups and their relatives, and the treatment-resistant group and their relatives, testing the assumption of equal means. If the assumption of normality was not met, the Mann-Whitney test was used. Fisher's exact test and chi-squared test were used to assess the association of categorical variables with frequency distribution. We also present the measurement of vitamin D levels in the groups based on demographic variables, ESR and CRP, and behavioral variables using the Mann-Whitney test.

## Results

The mean age in the treatment-responsive patients (group 1) was 45.88±18.51, and in the treatment-resistant patients (group 3) was 41.30±13.01 years ($P=0.33$). The mean serum levels of Vitamin D in the group 1 was 24.96±9.66 ng/mL and in the group 3 was 27.70±12.28 ng/mL, which showed that there is no significant relationship between ulcerative colitis and serum vitamin D levels between the groups studied ($P=0.41$). The size of the body mass index (BMI) in all four groups was in the range of 18.5–24.9. The The mean serum levels of CRP in group 1 was 18.42±28.30, group 2 was 5.79±0.60, group 3 was 16.57±25.84 and group 4 was 5.83±0.76. Demographic information, risk factors, vitamin D levels, and other variables in all four groups are given in Table 1.

The analysis of the results of blood parameters (CBC & ESR) in ulcerative colitis patients (groups 1 and 3) showed that the mean (Std. dev.) hemoglobin (HB) in the group1 is 13.80 (2.24) and in the group3 is 12.37 (2.10). A significant relationship was observed between hemoglobin levels and ulcerative colitis, with a *P*-value of 0.029. Also, the frequency of people whose ESR results were positive was 10 (%41.67) in group 1 and 12 (%52.17) in group 3, and therefore there was no significant relationship between the level of ESR and UC (*P*-value: 0.47). Table 2 displays the results of the analysis of blood parameters, including CBC and ESR, for groups 1 and 3.

Analysis of vitamin D concentrations, presented as mean (SD), in relation to BMI revealed the following trends: in groups 1 and 2, individuals with a BMI of less than 18.5 had the highest vitamin D levels. Conversely, in group 3, individuals with a BMI between 18.5 and 24.9 had the highest vitamin D levels, while in group 4, individuals with a BMI ranging from 25.0 to 29.9 showed the highest vitamin D concentrations.

For individuals consuming high-fat foods, mean (SD) vitamin D levels were 23.54 (8.35) in group 1 and 26.28 (12.77) in group 3. For those with positive CRP results, the mean (SD) vitamin D levels were 24.29 (8.22) in group 1 and 27.56 (12.73) in group 3.

Overall, analysis of vitamin D levels in relation to demographic variables, blood parameters, and other risk factors showed no significant relationships in any of the four groups. In addition, when vitamin D levels were examined by type of ulcerative colitis, the highest mean (SD) vitamin D levels in groups 1 and 3 were associated

**Table 1** Demographic information, risk factors, vitamin D levels, and other variables in all 4 groups

| Variable | Group 1 *n* (%) | Group 2 *n* (%) | *P*-Value[a] | Group 3 *n* (%) | Group 4 *n* (%) | *P*-Value[b] | *P*-Value [c] |
|---|---|---|---|---|---|---|---|
| **Age, mean (SD)** | 45.88 (18.51) | 37.29 (9.62) | 0.052† | 41.30 (13.01) | 40.96 (13.01) | 0.928† | 0.331† |
| **Sex** | | | | | | | |
| Male | 17 (70.83) | 17 (70.83) | 1.00* | 14 (60.87) | 14 (60.87) | 1.00* | 0.547* |
| Female | 7 (29.17) | 7 (29.17) | | 9 (39.13) | 9 (39.13) | | |
| **Residence** | | | | | | | |
| Rural | 5 (20.83) | 3 (12.50) | 0.701* | 8 (34.78) | 8 (34.78) | 1.00* | 0.341* |
| Urban | 19 (79.17) | 21 (87.50) | | 15 (65.22) | 15 (65.22) | | |
| **BMI** | | | | | | | |
| < 18.5 | 4 (16.67) | 3 (12.50) | 0.491* | 2 (8.70) | 2 (8.70) | 0.505* | 0.506* |
| 18.5–24.9 | 12 (50.00) | 8 (33.33) | | 15 (65.22) | 11 (47.83) | | |
| 25.0-29.9 | 6 (25.00) | 8 (33.33) | | 6 (26.09) | 10 (43.48) | | |
| > 30.0 | 2 (8.33) | 5 (20.83) | | 0 (0.00) | 0 (0.00) | | |
| **CRP** | | | | | | | |
| < 6 | 7 (29.17) | 10 (41.67) | 0.547* | 5 (21.74) | 11 (47.83) | 0.120* | 0.740* |
| ≥ 6 | 17 (70.83) | 14 (58.33) | | 18 (78.26) | 12 (52.17) | | |
| **Eating high-fat food** | | | | | | | |
| No | 11 (45.83) | 8 (33.33) | 0.556* | 5 (21.74) | 6 (26.09) | 1.00* | 0.125* |
| Yes | 13 (54.17) | 16 (66.67) | | 18 (78.26) | 17 (73.91) | | |
| **Seafood consumption** | | | | | | | |
| No | 5 (20.83) | 5 (20.83) | 1.00* | 3 (13.04) | 2 (8.70) | 1.00* | 0.701* |
| Yes | 19 (79.17) | 19 (79.17) | | 20 (86.96) | 21 (91.30) | | |
| **Taking vitamin D tablets** | | | | | | | |
| No | 12 (50.00) | 11 (45.83) | 0.773** | 7 (30.43) | 14 (60.87) | 0.075* | 0.238* |
| Yes | 12 (50.00) | 13 (54.17) | | 16 (69.57) | 9 (39.13) | | |
| **Consumption of local dairy products** | | | | | | | |
| No | 1 (4.17) | 2 (8.33) | 1.00* | 1 (4.35) | 3 (13.04) | 0.608* | 1.00* |
| Yes | 23 (95.83) | 22 (91.67) | | 22 (95.65) | 20 (86.96) | | |
| **History of COVID-19** | | | | | | | |
| No | 13 (54.17) | 13 (54.17) | 1.00** | 11 (47.83) | 13 (56.52) | 0.555** | 0.773** |
| Yes | 11 (45.83) | 11 (45.83) | | 12 (52.17) | 10 (43.48) | | |
| **Vitamin D (ng/mL), mean (SD)** | 24.96(9.66) | 21.83(7.81) | 0.325‡ | 27.70(12.28) | 25.78 (9.94) | 0.716‡ | 0.405‡ |

†: From t-test; *: From Fisher's exact test; ‡: From Mann–Whitney test; **: From Pearson's chi-squared; a: *P*-value from the comparison of Treatment and Relatives of the Treated group; b: *P*-value from the comparison of Treatment-resistant and Relatives of the Treatment-resistant group; c: *P*-value from the comparison of Treatment and Treatment-resistant group

**Table 2** The results of blood parameters (CBC & ESR) in two patients groups 1 and 3

| Variable | Group 1; *N* = 24; Mean (Std. dev.) | Group 3; *N* = 23; Mean (Std. dev.) | *P*-Value |
|---|---|---|---|
| WBC | 7.31 (1.65) | 7.05 (1.97) | 0.632‡ |
| RBC | 4.83 (0.54) | 4.61 (0.48) | 0.200‡ |
| HB | 13.80 (2.24) | 12.37 (2.10) | 0.029† |
| HCT | 41.73 (5.32) | 38.90 (4.81) | 0.063† |
| MCV | 85.61 (5.18) | 83.73 (7.41) | 0.198‡ |
| MCH | 28.27 (3.13) | 27.47 (4.06) | 0.371‡ |
| MCHC | 32.88 (2.16) | 32.10 (1.95) | 0.070‡ |
| PLT | 289.25 (82.97) | 317.04 (98.85) | 0.301† |
| Nut | 59.21 (6.69) | 60.70 (7.44) | 0.474† |
| Lym | 36.25 (6.93) | 32.43 (8.25) | 0.092† |
| ESR | | | |
| Negative; n (%) | 14 (58.33) | 11 (47.83) | 0.471** |
| Positive; n (%) | 10 (41.67) | 12 (52.17) | |

‡: From Mann–Whitney test; †: From t-test; **: From Pearson's chi-squared

with extensive colitis, while the lowest levels were associated with distal colitis (Tables 3 and 4).

## Discussion

Vitamin D is a lipid-soluble secosteroid hormone attributed to an immunomodulatory function [25]. The prevalence of vitamin D deficiency among patients with IBD has been reported between 22 and 63%. Many studies point to it as one of the environmental factors that play a role in IBD disease [26, 27]. In our study, the Vitamin D; Mean (Std. dev.) in treated and resistant ulcerative colitis patients was 24.96 (9.66) and 27.70 (12.28 ( respectively this amount was insufficient, so in the group of their first-degree relatives, who were the control group for them, it was also 21.83 (7.81) and 25.78 (9.94). Therefore, their analysis showed that there is no significant relationship between vitamin D and those who had ulcerative colitis (*P*-Value: 0.40), so even the data analysis of people

**Table 3** The analysis of serum levels of vitamin D with demographic characteristics and variables in all 4 groups

| | Vitamin D serum levels Mean (Std. dev.) | | | Vitamin D serum levels Mean (Std. dev.) | | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **Group 1** | **Group 2** | ***P*-Value[a]** | **Group 3** | **Group 4** | ***P*-Value[b]** | ***P*-Value[c]** |
| **Sex** | | | | | | | |
| Male | 24.18 (8.73) | 22.65 (8.74) | 0.534 | 26.71 (12.49) | 24.43 (9.89) | 0.872 | 0.604 |
| Female | 26.86 (12.19) | 19.86 (4.85) | 0.564 | 29.22 (12.53) | 27.89 (10.23) | 0.658 | 0.670 |
| **Residence** | | | | | | | |
| Rural | 20.00 (2.65) | 20.00 (4.36) | 0.881 | 25.13 (15.71) | 23.88 (8.10) | 0.832 | 0.883 |
| Urban | 26.26 (10.44) | 22.10 (8.22) | 0.289 | 29.07 (10.39) | 26.80 (10.92) | 0.560 | 0.357 |
| **BMI** | | | | | | | |
| < 18.5 | 27.00 (12.11) | 28.00 (8.19) | 0.593 | 19.50 (0.71) | 17.50 (4.95) | 1.00 | 0.240 |
| 18.5–24.9 | 25.50 (10.41) | 19.13 (3.91) | 0.278 | 29.00 (14.59) | 26.09 (9.36) | 0.835 | 0.574 |
| 25.0-29.9 | 23.67 (9.33) | 24.38 (10.34) | 0.647 | 27.17 (5.95) | 27.10 (11.14) | 0.745 | 0.470 |
| > 30.0 | 21.50 (4.95) | 18.40 (5.68) | 0.430 | - | - | - | - |
| **Eating high-fat food** | | | | | | | |
| No | 26.64 (11.19) | 23.50 (11.26) | 0.407 | 32.80 (9.73) | 30.67 (11.72) | 0.927 | 0.391 |
| Yes | 23.54 (8.35) | 21.00 (5.66) | 0.675 | 26.28 (12.77) | 24.06 (8.99) | 0.881 | 0.410 |
| **Seafood consumption** | | | | | | | |
| No | 24.60 (11.67) | 25.00 (13.06) | 0.916 | 20.33 (10.02) | 24.50 (4.95) | 0.767 | 0.882 |
| Yes | 25.05 (9.43) | 21.00 (6.04) | 0.305 | 28.80 (12.42) | 25.90 (10.36) | 0.505 | 0.382 |
| **Taking vitamin D tablets** | | | | | | | |
| No | 23.50 (9.42) | 19.09 (3.78) | 0.237 | 24.14 (6.47) | 27.64 (11.16) | 0.524 | 0.471 |
| Yes | 26.42 (10.09) | 24.15 (9.60) | 0.764 | 29.25 (14.00) | 22.89 (7.34) | 0.349 | 0.576 |
| **Consumption of local dairy products** | | | | | | | |
| No | 35.00 (0.00) | 17.50 (0.71) | 0.221 | 20.00 (0.00) | 31.33 (15.53) | 0.655 | 0.317 |
| Yes | 24.52 (9.63) | 22.23 (8.05) | 0.608 | 28.05 (12.45) | 24.95 (9.13) | 0.528 | 0.280 |
| **History of COVID-19** | | | | | | | |
| No | 24.15 (10.46) | 21.08 (6.46) | 0.486 | 21.82 (8.34) | 26.46 (10.18) | 0.190 | 0.884 |
| Yes | 25.91 (9.04) | 22.73 (9.40) | 0.575 | 33.08 (13.12) | 24.90 (10.09) | 0.129 | 0.138 |
| **CRP** | | | | | | | |
| < 6 | 26.57 (13.16) | 21.90 (7.08) | 0.590 | 28.20 (11.86) | 28.73 (8.45) | 0.459 | 0.935 |
| ≥ 6 | 24.29 (8.22) | 21.79 (8.55) | 0.413 | 27.56 (12.73) | 23.08 (10.77) | 0.181 | 0.418 |
| **ESR (*N* = 47)** | | - | - | - | - | - | - |
| Negative | 26.00 (9.96) | - | - | 27.36 (13.44) | - | - | 0.783 |
| Positive | 23.50 (9.55) | - | - | 28.00 (11.72) | - | - | 0.234 |
| **Ulcerative colitis type** | | | | | | | |
| Distal colitis | 18 (1.41) | - | - | 20 (0.00) | - | - | 0.221 |
| Extensive colitis | 40 (7.07) | - | - | 35.80 (16.13) | - | - | 0.699 |
| Left colitis | 25.33 (10.31) | - | - | 21 (0.00) | - | - | 0.727 |
| Pancolitis | 23.18 (8.12) | - | - | 26.06 (10.96) | - | - | 0.519 |

‡: From Mann–Whitney test; a: *P*-value from the comparison of Treatment and Relatives of the Treated group; b: *P*-value from the comparison of Treatment-resistant and Relatives of the Treatment-resistant group; c: *P*-value from the comparison of Treatment and Treatment-resistant group

who consumed vitamin D also did not have a significant relationship (*P*-Value: 0.50). The analysis of CRP and ESR data with vitamin D level also showed that there is no statistically significant relationship between them. However, from a clinical point of view, vitamin D levels are low among people whose CRP and ESR results are positive. Although studies state that there is an inverse relationship between BMI and vitamin D levels, in our study there was no statistically significant relationship between them among the 4 studied groups. The results of our study were consistent with some studies and inconsistent with others. The study of Daniel et al. [28] showed that there is no significant relationship between ulcerative colitis and vitamin D level, even between blood parameters (CRP), there was no significant relationship with it, although their results were similar to ours, the strength of our study is the type of population studied. Because in our study, the control subjects were first-degree relatives of the sick subjects, who had the same environmental conditions as the sick subjects. In a retrospective study, the average vitamin D level in individuals with ulcerative colitis was found to be 17.1 ± 9.7, while in

**Table 4** The results of vitamin D analysis with CBC in two groups 1 and 3

| Pearson Correlations Coefficient | | | | |
|---|---|---|---|---|
| **Variable** | **Spearman's rho of Vitamin D in group 1** | ***P*-Value[a]** | **Spearman's rho of Vitamin D in group 3** | ***P*-Value[b]** |
| White Blood Cell count | -0.08 | 0.69 | 0.299 | 0.16 |
| Red Blood Cell count | -0.33 | 0.11 | 0.405 | 0.05 |
| Hemoglobin | -0.153 | 0.47 | 0.102 | 0.64 |
| Hematocrit | -0.209 | 0.32 | 0.252 | 0.24 |
| Mean Corpuscular Volume | 0.178 | 0.40 | -0.04 | 0.84 |
| Mean Corpuscular Hemoglobin | 0.119 | 0.57 | -0.14 | 0.51 |
| Mean Corpuscular Hemoglobin Concentration | 0.08 | 0.70 | -0.236 | 0.27 |
| Platelet count | 0.107 | 0.61 | 0.40 | 0.05 |
| Neutrophils | -0.282 | 0.18 | -0.35 | 0.10 |
| Lymphocytes | 0.17 | 0.41 | 0.06 | 0.76 |

a: *P*-value from the comparison of Variable Vitamin D in group 1

b: *P*-value from the comparison of Variable Vitamin D in group 3

healthy individuals, it was 20.4±7.0 [29], although the average level of vitamin D in this study was lower than our study, like our study, there was no significant relationship between vitamin D level and ulcerative colitis. A strength of our study was the selection of the control group. The control group was a family member who had the same environmental and nutritional conditions as the patients. In another cross-sectional study, the average serum level of vitamin D in people with ulcerative colitis was 33.1±8.3, and despite the higher level of vitamin D compared to our study, there was no significant relationship between vitamin D and ulcerative colitis, and also, there was no significant relationship between ESR, CRP, and BMI parameters and vitamin D levels [30]. In a separate study, which differs from our findings, a notable association was observed between vitamin D levels and ulcerative colitis. In this study, the average serum level of vitamin D was reported as 10.32±4.46 in the patient group, compared to 12.87±4.40 in the control group. Although the control group also had low serum vitamin D levels, the study still demonstrated a significant relationship between vitamin D levels and ulcerative colitis [31]. The point that was different from our study in this study was the matching of patients and controls in terms of age and gender, and maybe this difference in results is related to this point. In other studies, there was a significant relationship between the serum level of vitamin D and ulcerative colitis [32, 33]. Overall, while our study did not find a significant relationship between serum vitamin D levels and ulcerative colitis and these results were consistent with several studies and contradicted with several others, the point that exists and should be taken into account is this, Although vitamin D plays a role in reducing inflammation and modulating the immune system, its role in many diseases, including digestive diseases, should still be investigated and discussed. Although vitamin D deficiency is common in people with IBD, as we mentioned, it is not clear whether this is a nutritional cause or a consequence of the disease because in our study the control subjects, which included patients with treated colitis and There were no patients who were first-degree relatives of the patients themselves, and their serum vitamin D levels were also low. Therefore, it seems that in addition to the vitamin D factor, other environmental factors should be investigated simultaneously in these patients, and the role of this vitamin in the pathogenesis of ulcerative colitis should also be studied.

## Conclusion

Despite the low serum concentration of vitamin D in individuals with ulcerative colitis, no significant relationship was found between them, and no meaningful association was observed between vitamin D levels and parameters such as ESR, CRP, and BMI. The impact of other factors, including underlying diseases, gender, ethnicity, age, and genetic susceptibility, warrants further investigation. Nevertheless, the reduction in serum levels of this vitamin in these patients remains concerning. So far, extensive studies have demonstrated that vitamin D plays a crucial role in regulating the mucosal immune system and overall immunity, which in turn affects intestinal inflammation [34]. The role of vitamin D-mediated pathways in maintaining intestinal homeostasis has also been extensively studied in experimental models [34]. Concurrently, randomized controlled trials involving patients with ulcerative colitis indicate that vitamin D has a protective effect against disease progression and suggest that vitamin D supplementation may lead to more favorable disease courses [34]. Well-designed therapeutic trials are needed to determine whether vitamin D supplementation can restore the gut microbiome to mitigate chronic inflammation and establish an appropriate dosage [34]. There is a significant need for comprehensive evaluations of the effects of vitamin D concentration on intestinal irritation, illness activity and progress, and whether vitamin D insufficiency is related with particular clinical phenotypes.

**Acknowledgements**

The authors express their gratitude to the Vice-chancellor of Research of Hamadan University of Medical Sciences, Hamadan, Iran, for advocating this research.

**Author contributions**
AA, MYA, RY: data curtain. AM, MKM: formal analysis. LS, FS: investigation. MYA, RY: writing – original draft. A.A, BN help edit the text of the article. All authors studied and approved the content of the present manuscript and participated in revising the paper.

**Funding**
This study was funded by the Vice-chancellor of Research and Technology of Hamadan University of Medical Sciences, Hamadan, Iran, under Grant number: 140110279103.

**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
This study was approved by the Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran (Ethic approval code: IR.UMSHA.REC.1401.787). Written informed consent from the subjects of the study has been obtained. All methods were conducted following relevant guidelines and regulations.

**Consent to publish**
Not applicable.

**Competing interests**
The authors declare no competing interests.

**Author details**
[1]Infectious Disease Research Center, Avicenna Institute of Clinical Sciences, Avicenna Health Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[3]Liver and Digestive Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
[4]Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
[5]Institute of Virology, Helmholtz Munich, German Research Centre for Environmental Health, Neuherberg, Germany
[6]Chair of Prevention of Microbial Infectious Diseases, Central Institute of Disease Prevention, School of Life Sciences, Technical University of Munich, Freising, Germany

Received: 21 March 2024 / Accepted: 10 December 2024
Published online: 18 December 2024

**References**

1. Matsuoka K, Kobayashi T, Ueno F, Matsui T, Hirai F, Inoue N, et al. Evidence-based clinical practice guidelines for inflammatory bowel disease. J Gastroenterol. 2018;53(3):305–53.
2. Segal JP, LeBlanc JF, Hart AL. Ulcerative colitis: an update. Clin Med (Lond). 2021;21(2):135–9.
3. Du L, Ha C. Epidemiology and Pathogenesis of Ulcerative Colitis. Gastroenterol Clin North Am. 2020;49(4):643–54.
4. Loscalzo J, Fauci A, Kasper D, Hauser S, Longo D, Jameson JL, Editors. Harrison's principles of Internal Medicine, 21e. New York, NY: McGraw-Hill Education; 2022.
5. Langan RC, Gotsch PB, Krafczyk MA, Skillinge DD. Ulcerative colitis: diagnosis and treatment. Am Fam Physician. 2007;76(9):1323–30.
6. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012;142(1):46–e54. 42; quiz e30.
7. Rebelos E, Tentolouris N, Jude E. The Role of Vitamin D in Health and Disease: a narrative review on the mechanisms linking vitamin D with Disease and the effects of Supplementation. Drugs. 2023;83(8):665–85.
8. Raman M, Milestone AN, Walters JR, Hart AL, Ghosh S. Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer. Th Adv Gastroenterol. 2011;4(1):49–62.
9. Ao T, Kikuta J, Ishii M. The effects of vitamin D on Immune System and Inflammatory diseases. Biomolecules. 2021;11(11).
10. Barbalho SM, Goulart RA, Gasparini RG. Associations between inflammatory bowel diseases and vitamin D. Crit Rev Food Sci Nutr. 2019;59(8):1347–56.
11. Fletcher J, Cooper SC, Ghosh S, Hewison M. The role of vitamin D in inflammatory bowel disease: mechanism to management. Nutrients. 2019;11(5).
12. Kellermann L, Jensen KB, Bergenheim F, Gubatan J, Chou ND, Moss A, et al. Mucosal vitamin D signaling in inflammatory bowel disease. Autoimmun Rev. 2020;19(11):102672.
13. Ghaseminejad-Raeini A, Ghaderi A, Sharafi A, Nematollahi-Sani B, Moossavi M, Derakhshani A, et al. Immunomodulatory actions of vitamin D in various immune-related disorders: a comprehensive review. Front Immunol. 2023;14:950465.
14. Yamamoto E, Jørgensen TN. Immunological effects of vitamin D and their relations to autoimmunity. J Autoimmun. 2019;100:7–16.
15. Illescas-Montes R, Melguizo-Rodríguez L, Ruiz C, Costela-Ruiz VJ. Vitamin D and autoimmune diseases. Life Sci. 2019;233:116744.
16. Limketkai BN, Singla MB, Rodriguez B, Veerappan GR, Betteridge JD, Ramos MA, et al. Levels of vitamin D are low after Crohn's disease is established but not before. Clin Gastroenterol Hepatol. 2020;18(8):1769–e761.
17. Limketkai BN, Bechtold ML, Nguyen DL. Vitamin D and the Pathogenesis of Inflammatory Bowel Disease. Curr Gastroenterol Rep. 2016;18(10):52.
18. Myint A, Sauk JS, Limketkai BN. The role of vitamin D in inflammatory bowel disease: a guide for clinical practice. Expert Rev Gastroenterol Hepatol. 2020;14(7):539–52.
19. El Amrousy D, El Ashry H, Hodeib H, Hassan S. Vitamin D in Children with Inflammatory Bowel Disease: a Randomized Controlled Clinical Trial. J Clin Gastroenterol. 2021;55(9):815–20.
20. Karimi S, Tabataba-Vakili S, Ebrahimi-Daryani N, Yari Z, Karimi A, Hedayati M, et al. Inflammatory biomarkers response to two dosages of vitamin D supplementation in patients with ulcerative colitis: a randomized, double-blind, placebo-controlled pilot study. Clin Nutr ESPEN. 2020;36:76–81.
21. Tan B, Li P, Lv H, Yang H, Li Y, Li J, et al. Treatment of vitamin D deficiency in Chinese inflammatory bowel disease patients: a prospective, randomized, open-label, pilot study. J Dig Dis. 2018;19(4):215–24.
22. Li J, Chen N, Wang D, Zhang J, Gong X. Efficacy of vitamin D in treatment of inflammatory bowel disease: a meta-analysis. Med (Baltim). 2018;97(46):e12662.
23. Wallace C, Gordon M, Sinopoulou V, Limketkai BN. Vitamin D for the treatment of inflammatory bowel disease. Cochrane Database Syst Rev. 2023;10(10):Cd011806.
24. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266–81.
25. Limketkai BN, Mullin GE, Limsui D, Parian AM. Role of vitamin D in inflammatory bowel disease. Nutr Clin Pract. 2017;32(3):337–45.
26. Frigstad SO, Høivik M, Jahnsen J, Dahl SR, Cvancarova M, Grimstad T, et al. Vitamin D deficiency in inflammatory bowel disease: prevalence and predictors in a Norwegian outpatient population. Scand J Gastroenterol. 2017;52(1):100–6.
27. Sadeghian M, Saneei P, Siassi F, Esmaillzadeh A. Vitamin D status in relation to Crohn's disease: Meta-analysis of observational studies. Nutrition. 2016;32(5):505–14.
28. Caviezel D, Maissen S, Niess JH, Kiss C, Hruz P. High prevalence of vitamin D Deficiency among patients with inflammatory bowel disease. Inflamm Intest Dis. 2018;2(4):200–10.
29. Hausmann J, Kubesch A, Amiri M, Filmann N, Blumenstein I. Vitamin D Deficiency is Associated with increased disease activity in patients with inflammatory bowel disease. J Clin Med. 2019;8(9).
30. Sharifi A, Nedjat S, Vahedi H, Veghari G, Hosseinzadeh-Attar MJ. Vitamin D status and its relation to inflammatory markers in patients with mild to Moderate Ulcerative Colitis. Middle East J Dig Dis. 2018;10(2):84–9.
31. Tan B, Li P, Lv H, Li Y, Wang O, Xing XP, et al. Vitamin D levels and bone metabolism in Chinese adult patients with inflammatory bowel disease. J Dig Dis. 2014;15(3):116–23.
32. Hormati A, Azad M, Mohammadbeigi A, Maghsoudi V, Rezvan S, Mokhtarian MH, et al. Evaluation of the relationship between serum vitamin D levels and disease recurrence in patients with ulcerative colitis. Tehran Univ Med J. 2021;79(6):468–73.

33. Law AD, Dutta U, Kochhar R, Vaishnavi C, Kumar S, Noor T, et al. Vitamin D deficiency in adult patients with ulcerative colitis: prevalence and relationship with disease severity, extent, and duration. Indian J Gastroenterol. 2019;38(1):6–14.
34. Triantos C, Aggeletopoulou I, Mantzaris GJ, Mouzaki A. Molecular basis of vitamin D action in inflammatory bowel disease. Autoimmun Rev. 2022;21(8):103136.

## Publisher's note