Observational Study

Medicine

OPEN

An assessment of serum vitamin B12 and folate in patients with Crohn's disease

Sabiye Akbulut, MD[a,*]

Abstract

Crohn's disease is a chronic inflammatory condition that can involve any area in the gastrointestinal tract often involving the distal ileum where vitamin B12 is specifically absorbed. The aim of this study was to ascertain serum vitamin B12 and folate levels in order to investigate the correlation among these vitamin levels and disease activation, localization, duration and age at the onset of the disease.

Study population included 103 patients with Crohn's disease and a healthy control group of 114 individuals. C-reactive protein, vitamin B12, folate levels were studied along with hemogram analyses. The results were evaluated in statistical comparisons. While serum vitamin B12 levels and serum folate levels were 161.9 ± 63.2(73–496) pg/mL and 4.9 ± 1.4(1.2–9.4) ng/mL in the Crohn's patient group respectively, they were 321.7 ± 126.3(85–680) pg/mL and 7.6 ± 3.8(3–25.1) ng/mL in the control group respectively. Vitamin B12 and folate levels were distinctly lower in patients with Chron's disease than those of the control group ($P < .001$). The intragroup analysis of the patient group revealed that low vitamin B12 levels were significantly lower in the moderate group classified according to the Crohn's Disease Activity Index ($P < .001$), along with those in the L1 group with terminal/distal ileal involvement ($P < .001$).

Vitamin B12 and folate deficiencies are quite prevalent in patients with Crohn's disease while this condition can lead to various complications and they prove to be important risk factors associated especially with thrombosis and its complications. Patients must be regularly followed-up for vitamin B12 and folate levels to supplement them where needed.

Abbreviations: AZA = azathioprine, CD = Crohn's disease, CDAI = Crohn's disease activity index, CRP = C-reactive protein, FA = folic acid, IBD = inflammatory bowel disease.

Keywords: Crohn's disease, folate, inflammatory bowel disease, vitamin B12

1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal system which progresses with remissions and relapses and has 2 major types: ulcerative colitis and Crohn's disease (CD). While ulcerative colitis has limited involvement in the large intestine; CD is a chronic, transmural and inflammatory disease that can be involved in any part of the gastrointestinal tract.[1] The disease emerges with inflammation and then can often lead to fibrosis when the inflammation becomes transmural while it can cause clinically obstructive symptoms. When fibrosis further progresses and the inflammation reaches the serosa, microperforations and fistulas develop. Although CD can involve any area in the gastrointestinal tract, it usually involves the terminal ileum and the colon.[2,3] If the intestinal inflammation in CD is not treated, it can cause such intestinal complications as strictures, fistulas, and abscess.[3]

Although the definitive cause of CD is yet to be known, researchers have suggested that it develops as a result of the interaction among genetic factors, microbial exposure, epigenetic factors, immune response, and environmental factors.[4,5] The risk factors that affect the clinical progress of Chron's disease include disease onset at a young age, extensive anatomical involvement, perianal disease, stricture or penetrating disease, deep ulcers, and history of surgical resection.[6]

The area where CD most often involves is the distal ileum or terminal ileum where vitamin B12 is specifically absorbed. Vitamin B12 deficiency and malabsorption can, therefore, be expected in CD.[7] Folate or folic acid (FA) is an essential nutrient and has a homocysteine-lowering effect.[8] Patients may develop macrocytic anemia, hyperhomocysteinemia, neurological and psychiatric disorders if they suffer from vitamin B12 and folate deficiencies. Further, hyperhomocysteinemia associated with vitamin B12 and folate deficiency in such patients is recognized as a risk factor for thrombosis.[9]

The aim of this study was to identify the factors bringing about vitamin B12 and folate deficiencies in CD by ascertaining B12 and folate levels in patients with CD.

2. Materials and Methods

This study was a single-center retrospective study conducted at the University of Health Sciences, Kartal Koşuyolu High

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Gastroenterology, University of Health Sciences, Kartal Koşuyolu High Specialty Training and Research Hospital, Istanbul, Turkey.

** Correspondence: Sabiye Akbulut, Department of Gastroenterology, University of Health Sciences, Kartal Koşuyolu High Specialty Training and Research Hospital, Istanbul 34865, Turkey (e-mail: sabiye4@hotmail.com).*

How to cite this article: Akbulut S. An assessment of serum vitamin B12 and folate in patients with Crohn's disease. Medicine 2022;101:50(e31892).

Received: 12 November 2021 / Received in final form: 26 October 2022 / Accepted: 27 October 2022

http://dx.doi.org/10.1097/MD.0000000000031892

Specialty Training and Research Hospital. A total of 103 patients, who had been followed up and treated at our gastroenterology outpatient clinic between September, 2016 and January, 2021 having been diagnosed with CD based on clinical, endoscopic, laboratory, histologic, and radiographic data, were included in the study. Patients' data were collected at the hospital's outpatient clinic as the patients presented to outpatient visits and the hospital's medical electronic records within the scope of the analyses performed for the study.

The study protocol was approved by the University of Health Sciences, Kartal Koşuyolu High Specialty Training and Research Hospital's Committee of Ethics (2016-KAEK-112).

2.1. Subjects

Two groups were designated for the study as the patient group and the control group, while pregnant patients and those who received vitamin B12 and FA supplements within the last 6 months in both groups were excluded from the study.

The control group consisted of healthy individuals who had presented to the hospital for checkups. These individuals did not have any previous history of gastrointestinal disease and history of surgical procedure and/or history of medication. Their physical examination results were normal.

In the CD patient group, data on age, sex, age at the onset of the disease, duration of the disease, disease localization, disease activation, laboratory parameters, medical treatments received, and ileal and/or colon resection histories were recorded for each patient.

While age at CD onset, disease localization, and CD behavior were recorded based on the Montreal classification,[10] CD activation was recorded according to the Crohn's disease activity index (CDAI).[11]

2.2. Criteria and definitions

The Montreal classification was used to ascertain CD characteristics in patients with Crohn's disease in this study. According to the classification done as per age at the onset of disease within the scope of this classification, the following were designated as A1 ≤ 16 years of age, A2 ages between 17 to 40, A3 > 40 years of age, while disease localization was identified as ileum (L1), colon (L2), ileocolon (L3), upper gastrointestinal (L4), and disease behavior as non-stricturing/non-penetrating, inflammatory (B1), stricturing (B2), penetrating (B3), perianal disease (P).

Crohn's Disease Activity Index (CDAI) was used to determine CD activation in the patient group. CDAI < 150 was assessed to be inactivity or remission, CDAI 150 to 219 as mild, CDAI 220 to 450 as moderate, and CDAI > 450 as severe disease. Disease activity was calculated according to the CDAI and recorded for each patient.

Fasting venous blood samples were collected from both groups to analyze hemogram, routine biochemical tests, C-reactive protein (CRP), vitamin B12 and folate levels. CRP was measured automatically with a nephelometric analyzer system. Plasma vitamin B12 and folate levels were measured by specific immunochemical methods.

Vitamin B12 and folate deficiencies were defined as per local laboratory criteria and serum vitamin B12 deficiency was set at ≤ 126 pg/mL, while folate deficiency was set at serum folate level < 3 ng/mL.

CRP inflammation indicator was recorded as a marker. Anemia diagnosis and macrocytosis were defined according to local laboratory criteria and international standards. Anemia in adults was set at < 12 gr/dL for (non-pregnant) women and at < 13 gr/dL for men, while macrocytosis was set at mean corpuscular volume (>98 fL).[12]

2.3. Statistical analysis

SPSS 15.0 for Windows software was used for statistical analyses. Descriptive statistics were presented in numbers and percentages for categorical variables, while they were presented in mean, standard deviation, minimum, maximum, median figures and in interquartile ranges for numeric variables. As the comparisons of numeric variables in independent groups did not meet the normal distribution conditions, the Mann-Whitney U test was used for comparisons between 2 groups, while the Kruskal-Wallis test was used for comparisons among more than 2 groups. Subgroup analyses were performed by the Mann-Whitney U test and interpreted with the Bonferroni correction. Rates in the groups were compared by the chi-square analysis. The statistical alpha significance level was set at $P < .05$.

3. Results

The population of the study consisted of a total of 103 patients 52 (% 50.5) of whom were male and 51 (% 49.5) were female, while a total of 114 individuals were allocated to the control group with 56 (%49.1) males and 58 (%50.9) females. There was no statistical significance as per sex between the groups ($P = .841$). The mean age was 46.4 ± 13.5 (19–76) years in the CD patient group, while it was 43.2 ± 13.2 (18–74) years in the control group bearing no statistical significance between the 2 groups ($P = .073$) (Table 1).

While the mean serum vitamin B12 and mean serum folate levels were 161.9 ± 63.2 (73–496) pg/mL and 4.9 ± 1.4 (1.2–9.4) ng/mL in the CD patient group respectively, the mean serum vitamin B12 and mean serum folate levels were 321.7 ± 126.3 (85–680) pg/mL and 7.6 ± 3.8 (3–25.1) ng/mL in the control group respectively. Statistically, the vitamin levels were significantly lower in the patient group than the control group ($P < .001$, $P < .001$). When the CD patient group was compared to the control group with regards to CRP and hemoglobin, it was observed that the mean CRP figure was significantly higher in the patient group than the control group ($P < .001$), while the patient group's hemoglobin values were significantly lower ($P < .001$) than those of the control group (Table 2).

The CDAI classification revealed that 63 (61.2%) of the patients were in remission defined as inactive patients. The number of patients with mild disease according to the CDAI was 24 (23.3%), while 16 (15.5%) were moderate. Vitamin B12 levels were found to be the lowest in the moderate group (mean vitamin B12 level: 120 pg/mL [87–126.25]). The mean vitamin B12 figure for the inactive group (mean vitamin B12 level: 167 pg/mL [136–208]) was found to be significantly higher than those of the active patients in the mild (mean vitamin B12 level: 125 pg/mL [99.75–168.5] and moderate [mean vitamin B12 level: 120 pg/mL [87–126.25] categories $P = .001$ $P < .001$]). There was, however, no statistically significant difference in the mean vitamin B12 levels of the mild and moderate patients ($P = .214$). In other words, vitamin B12 levels were significantly lower in those with CDAI > 150 and over in comparison to the inactive group ($P = .001$ $P < .001$) (Fig. 1), (Table 3). When we evaluated the folate levels according to CDAI, no significant difference was observed between the active and inactive groups in terms of folate levels ($P = .729$) (Table 3).

There was no statistically significant difference among the groups when they were compared with regards to vitamin B12 and folate levels according to the duration of the disease as well ($P = .554$) ($P = .952$) (Table 3).

When the patient group was analyzed as per age at the time of CD diagnosis it was observed that there was no statistically significant difference among the groups ($P = .809$), although vitamin B12 levels were found to be lower in those diagnosed at an early age (<16 years of age with vitamin B12 levels of 142.5 pg/mL, those diagnosed between 17–40 years of age with vitamin B12 levels of 157 pg/mL, and those diagnosed when they were

Table 1

Clinical characteristics of the patient and the control groups.

	Patient group (n = 103)	Control group (n = 114)	*P*
Mean age(yr)	46.4 ± 13.5	43.2 ± 13.2	.073
Sex			
Female	52 (50.5%)	56 (49.1%)	.841
Male	51 (49.5%)	58 (50.9%)	
CDAI			
Inactive	63 (61.2%)		
Mild	24 (23.3%)		
Moderate	16 (15.5%)		
Duration of disease			
0–5 yr	31 (30.1%)		
6–10 yr	25 (24.3%)		
>10 yr	47 (45.6%)		
Montreal Classification			
A1	4 (3.9%)		
A2	59 (57.3%)		
A3	40 (38.8%)		
L1	51 (49.5%)		
L2	12 (11.7%)		
L3	40 (38.8%)		
B1	67 (65%)		
B2	18 (17.5%)		
B3	13 (12.6%)		
Perineal disease	5 (4.9%)		
Upper gastrointestinal disease	0 (0%)		
Prior ileal or ileocolic resection	31 (30.1%)		
Treatment of CD			
5-ASA	52 (50.5%)		
AZA	28 (27.2%)		
Biologic + AZA	12 (11.7%)		
Biologic agents	6 (5.8%)		
Sulfasalazin	2 (1.9%)		
5-ASA + AZA	2 (1.9%)		
Sulfasalazin + AZA	1 (1%)		

ASA = 5-aminosalicylic acid, AZA = azathioprine, CD = Crohn's disease, CDAI = Crohn's disease activity index.

over 40 with vitamin B12 levels of 163 pg/mL). When we analyzed the folate levels in this group, no statistically significant difference was found between the groups ($P = .138$) (Table 3).

Our results showed that the group with the lowest vitamin B12 levels was Crohn's patients with L1 involvement according to the Montreal classification (mean vitamin B12 level: 124 pg/mL (99-161)). (Fig. 2), (Table 3).

There was a statistically significant difference in mean vitamin B12 figures in CDAI and localization groups within the CD group ($P < .001$ for both). When the CD group was investigated in terms of disease localization, it was observed that there was no patient with upper gastrointestinal involvement (L4) in our patient group. Yet, the number of patients with L2 colonic involvement was 12 (11.7%), the number of patients with L1 ileal involvement was 51 (49.5%), and the number of patients with L3 ileocolonic involvement was 40 (38.8%). The lowest mean vitamin B12 level of 124 pg/mL (99–161 pg/mL) was found in the L1 group with ileal involvement. CD behavior study in the CD patient group revealed that the patients with the inflammatory type identified as B1 made up the majority with 67 (65%) patients. The subgroup analysis of the mean vitamin B12 and folate values showed no statistically significant difference among the groups ($P = .350$, $P = .459$) (Table 3).

When the CD group was studied it was observed that 31 (%30.1) patients had received surgical procedures due to various CD-related complications. 6 of them had had colon resections and the remaining 25 had a history of ileal, ileocolonic resection. When the patients who had had surgical procedures and those who had not were compared with regards to vitamin B12 and folate levels, it was seen that there was no statistically significant difference between the 2 although vitamin B12 and folate levels were lower in patients with a history of surgical procedure ($P = .386$; $P = .708$) (Table 3).

When the CD group was studied according to the treatments they had received, it was seen that the vitamin B12 levels of the patients who had received biological agents were found to be significantly higher than those who had not ($P = .010$) (Table 3).

The number of patients with vitamin B12 deficiency (<126 pg/mL) in the CD group was 37 (35.9%), while it was 3 (2.6%) in the control group. Statistically vitamin B12 deficiency in the CD group was significantly higher than the control group ($P < .001$).

When the patient and control groups were statistically compared in terms of folate deficiency (<3 ng/mL), it was seen that there were 3 patients (2.9%) with folate deficiency in the Crohn's disease group while there was none with folate level of < 3 in the control group. Folate deficiency comparison between the Crohn's disease group and the control group revealed no statistically significant difference ($P = .105$) (Table 4).

4. Discussion

Vitamin B12 (cobalamin) is a water-soluble vitamin which is essential for DNA synthesis, maintenance of erythropoiesis, stability of the nervous system, and functioning of fat and carbohydrate metabolism.[13,14] As it cannot be synthesized by the human body, it needs to be derived from food, particularly from foods of animal origin.[2] The clinical manifestation of vitamin B12 deficiency is quite variable ranging from mild nonspecific symptoms to severe ones progressing with neurological and neuropsychiatric disorders depending on the severity of vitamin B12 deficiency.[13]

Folate, like vitamin B12, is a water-soluble nutrient that is essential for the human body and plays a role in homocysteine regulation. In humans, folate plays a very significant role in methyl metabolism while it is directly engaged in such critical mechanisms as DNA methylation.[8] Just like vitamin B12, folate is needed for erythropoiesis and nervous system functions as well.[15] Folate is found both in foods of animal origin and vegetables, and is absorbed in the proximal small intestine including the duodenum and the jejunum.[16]

Table 2

Laboratory results for the patient and control groups.

	Patient group		Control group		*P*
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
B12	161.9 ± 63.2 (73–496)	161 (120–185)	321.7 ± 126.3 (85–680)	302.5 (236.25–383.5)	<.001
FA	4.9 ± 1.4 (1.2–9.4)	4.6 (4–5.6)	7.6 ± 3.8 (3–25.1)	6.65 (4.85–9.1)	<.001
CRP	7.9 ± 11.7 (1.2–94)	4.08 (3.11–9.11)	1.7 ± 1.6 (0.07–9.71)	1.27 (0.64–2.43)	<.001
Hb	12.8 ± 1.8 (7.6–16.4)	13 (11.8–13.7)	14.0 ± 1.9 (8.9–18.3)	13.95 (12.8–15.2)	<.001
MCV	83.2 ± 7.7 (58.4–94.5)	84.8 (79.8–88)	85.5 ± 5.9 (60.9–105.4)	86.2 (82.9–88.5)	.110

CRP = C-reactive protein, FA = folic acid, Hb = hemoglobin, MCV = mean corpuscular volume.

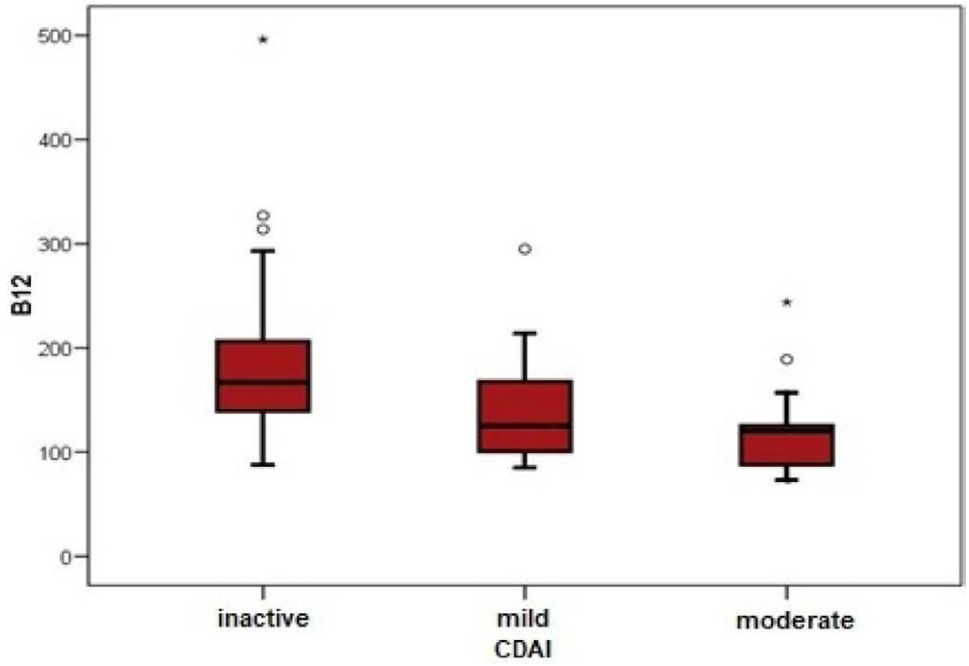

Figure 1. The minimum, maximum, 25–75% percentile, median B12 levels for the CDAI groups. CDAI = Crohn's disease activity index.

Macro and micronutrient deficiency in CD is quite often seen both in relapse and remission stages. Patients suffer from weight loss and particularly from decreased muscle mass and sarcopenia in cases of macronutrient deficiency-related malnutrition, which in turn elongates hospitalization time, delay in wound healing, and increased peri and postoperative complications and mortality rates. Micronutrient deficiency is also prevalent in remission and relapse stages in CD, the most common being iron, vitamin B12 and folate deficiencies among the micronutrients.[17]

There are studies in literature on the subject that have reported low vitamin B12 and folate levels in CD.[3,4,7,14,18–23] The results of our study, too, revealed that both vitamin B12 and folate levels were significantly lower in the CD group than those of the control group ($P < .001$). There are numerous factors bringing about vitamin B12 and folate deficiencies in CD which include common distal ileal involvement, long-term inflammation or fibrosis or stricture in the absorption area caused by the surgical resection of this segment, fistula development, and intestinal bacterial overgrowth.[3,16] Reduction in vitamin intake during the activation periods of the disease, intestinal malabsorption, and increase in need are also among the most common causes.[13,14] Moreover, inflammation of the distal ileum and disorders in the enterohepatic circulation of vitamin B12 also contribute to malabsorption.[24]

The situation is a bit different for folate. One might expect to see lesser folate deficiency in CD as it is usually absorbed in the duodenum and proximal jejunum. Malnutrition, malabsorption and increase in need can be listed among the causes of folate deficiency in active patients with CD rather than absorption.[16] Further, administration of medication for treatment like sulfasalazine and methotrexate that lower folate levels can lead to deficiency as well.[16,25]

When the CD group was analyzed within the scope of our study according to age at the onset of the disease, duration of disease, disease behavior and CDAI, we found that low vitamin levels were correlated with disease activity and disease localization. Patients with high CDAI ($P < .001$) and those with ileal involvement had significantly lower vitamin levels ($P < .001$). Patients who had received intestinal resection due to the disease ($P = .386$, $P = .708$), those who had been diagnosed at an early age (<16) ($P = .809$, $P = .138$), and those at the early stages of the disease, particularly those in the first 5 years ($P = .554$, $P = .952$), were found to have low vitamin levels but we found no statistically significant difference when the subgroups were compared.

CD is an inflammatory disease that progresses with relapses and remissions. The disease begins with inflammation and about 20% of the patients move from the inflammatory (B1) type on to the stricture (B2) type within a 5-year period as the years pass on usually with an increase in inflammation attacks and in disease activity.[16,26] Therefore, vitamin B12 deficiency can be seen more in those who contracted the disease at early ages. Although the results of our study revealed that the vitamin levels in patients who had received ileal or ileocolonic resection were lower than those who had not, no statistically significant difference was found between the 2 ($P = .386$). Researchers reported in a study that vitamin B12 malabsorption was observed in patients with ileal resection if the ileum was resected more than 50 to 60 centimeter.[19] We, however, were not able to have access to data revealing resection sizes in patients with ileal resection.

In our study, the number of patients with vitamin B12 deficiency (<126 pg/mL) in the CD group was 37 (35.9%), which was quite high in comparison to the control group ($P < .001$). And while the folate levels were lower than those of the control group, there was no significant difference with regards to folate deficiency ($P = .105$). Vitamin B12 and folate deficiencies may cause macrocytic anemia, hyperhomocysteinemia, neurological and psychiatric disorders while hyperhomocysteinemia is an important risk factor for thrombosis and thromboembolic complications.[9,27–29]

Risk of a thromboembolic event is higher in patients with IBD.[30] The incidence of systemic thromboembolism in IBD patients was reported to be 1% to 7% in clinical studies, while it was reported to be as high as 41% in postmortem studies. Thromboembolic complications seen in the course of the disease lead to increased patient morbidity and mortality.[31] Specifically during the activation phases of CD a hypercoagulable state occurs. Although the exact cause of this state remains unknown, researchers suggested that it was multifactorial. Increase in serum levels of plasminogen activator inhibitor, factors V and VIII and fibrinogen; decrease in factor V Leiden, factor XIII,

Table 3

B12 and FA levels in the disease characteristics of the CD group.

	n (%)	B12 Median (IQR)	FA Median (IQR)
CDAI			
Inactive	63 (61.2%)	167 (136–208)	4.6 (4–5.6)
Mild	24 (23.3%)	125 (99.75–168.5)	5 (4.125–5.675)
Moderate	16 (15.5%)	120 (87–126.25)	4.6 (3.25–5.725)
*P**		<.001	.729
Duration of disease			
0–5 yr	31 (30.1%)	154 (120–189)	4.6 (4–5.8)
6–10 yr	25 (24.3%)	162 (121–209)	4.5 (4.05–5.45)
>10	47 (45.6%)	161 (120–181)	4.5 (4–5.6)
*P**		.554	.952
Montreal classification			
A1	4 (3.9%)	142.5 (122.5–226.25)	6 (3.85–7.025)
A2	59 (57.3%)	157 (120–184)	4.5 (4.3–5.6)
A3	40 (38.8%)	163 (120.75–185)	4.6 (4.3–5.6)
*P**		.809	.138
L1	51 (49.5%)	124 (99–161)	4.6 (4–5.4)
L2	12 (11.7%)	163.5 (111.5–202.75)	4.3 (4–4.725)
L3	40 (38.8%)	176 (162.5–207.75)	4.8 (4.2–5.975)
*P**		<.001	.319
B1	67 (65%)	162 (122–194)	4.5 (4–5.6)
B2	18 (17.5%)	144.5 (117.7–195.75)	4.7 (3.95–5.4)
B3	13 (12.6%)	165 (111–180)	5.2 (4.45–6.1)
Perineal disease	5 (4.9%)	125 (103.5–143)	5 (4–7.05)
*P**		.35	.459
Resection			
Yes	31 (30.1%)	157 (119–185)	5 (4–5.8)
No	72 (69.9%)	162 (120.5–185)	4.55 (4–5.6)
*P**		.386	.708
5-ASA			
Yes	52 (50.5%)	161 (121.5–184.2)	4.3 (4–5.175)
No	51 (49.5%)	161 (120–201)	5.2 (4.25–5.85)
*P**		.797	.02
AZA			
Yes	28 (27.2%)	150 (119–187)	5.1 (4.3–5.8)
No	75 (72.8%)	161.5 (122–185)	4.3 (4–5.375)
*P**		0.642	0.052
Biologic agents			
Yes	6 (5.8%)	174.5 (161.7–220.7)	5.1 (4.175–6.2)
No	97 (94.2%)	150 (119.5–179.5)	4.5 (4–5.55)
*P**		0.01	0.203
Sulfasalazin			
Yes**	2 (1.9%)	219 (120–314)	5.3 (5.3–6)
No	101 (98.1%)	161 (120–184.75)	4.55 (4–5.6)
*P**			

ASA = 5-aminosalicylic acid, AZA = azathioprine, CD = Crohn's disease, CDAI = Crohn's disease activity index.

*CDAI Inactive vs mild L2 $P = .001$ Inactive vs moderate L3 $P < .001$ mild vs moderate $P = .214$ Localization L1 vs L2 $P = .089$ L1 vs L3 $P < .001$ L2 vs L3 $P = .152$.

**Insufficient sample excluded from the analysis.

antithrombin III, and proteins C and S in addition to spontaneous thrombocyte aggregation and thrombocytosis are seen.[32]

Folate is an essential co-factor in the metabolism of homocysteine-methionine, and folate deficiency may lead to hyperhomocysteinemie.[15] Hyperhomocysteinemia is quite common in patients with CD and plays a role in the pathogenesis of thrombotic episodes as a cofactor. Low cobalamin and particularly low folate levels prove to be an important factor in hyperhomocysteinemia development rather than disease activation. Maintaining normal levels of these vitamins in patients with CD is a primary factor in reducing the risk of thrombosis.[33]

Oral 5-aminosalicylates acid, antibiotics, immunosuppressive drugs like glucocorticoids and azathioprine (AZA) and biologic agents known as immunomodulators are used in the medical treatment of CD. Surgical treatment may be needed to initiate clinical remission in patients unresponsive to medical treatment or if there is an indication.[34] Today methotrexate and sulfasalazine, therapeutic agents that may cause folate deficiency, are rarely used.[16,25] The number of patients who were on sulfasalazine, which is an agent that might disrupt folate absorption, was 3 in our study and all had normal folate levels. While folate levels were found to be significantly low only in the 5-aminosalicylic acid receiving group for treatment ($P = .020$), vitamin B12 levels of patients receiving only biologic agents or dual therapy (biologic agent + AZA) were found to be significantly higher than those who did not ($P = .010$ $P = .006$) (Table 3). We believe that the reason for such results is that the treatment was effective in most patients receiving biologic agents and biologic agents + AZA and their CDAI were low with good vitamin B12 absorption from the mucosa.

The rate of malnutrition in Crohn's disease is quite high with a rate of 65 to 75%.[35] The severity of malnutrition is correlated with CD activity; the control of CD activity with a well-established treatment leads to an improvement in patients' nutritional state. In a study by Wiese et al, a CD patient receiving infliximab therapy was reported to have improved nutrition in parallel with reduced inflammation, gained weight and the patient's malnutrition status improved upon decreased lipolysis.[36]

Macrocytosis was not observed in any of the patients in the CD group although the patients' vitamin B12 and folate levels were low which, in turn, can be explained by the fact that microcytosis might have been masked by the coexistence of iron deficiency anemia in these patients.[37]

According to the European Society for Clinical Nutrition and Metabolism guidelines, patients with IBD should be regularly followed-up for micronutrient deficiencies and these vitamin deficiencies should be adequately replaced in order to maintain remission and reduce the severity of inflammation. Studies in literature also reported that serum vitamin profile could be used as a complementary biomarker, along with serum and stool inflammatory biomarkers, to assess disease activity.[38] Further, nutrigenomics studies associated micronutrients like vitamin D, vitamin E, calcium, FA, retinol and nicotinic acid that played a role in gene expression with reduced DNA damage and recommended that they be administered at an adequate dose for the treatment of patients with CD as they played a role in inflammation.[35]

Our study has some limitations; first is that it has a retrospective design in a single center. The second is the relatively low number of patients and controls. Third, although vitamin B12, folate and homocysteine levels were studied together in most of the studies on this subject in the literature, homocysteine levels were not evaluated in our study.

Consequently, the results of our study revealed that vitamin B12 and folate deficiency levels in patients with Crohn's disease were higher than those of the control group. Vitamin levels were also low in patients with high CDAI, those with distal ileal involvement, those diagnosed at an early age, and those who were within the 1st 5 years of the disease. Regular Vitamin B12 and folate level checks, necessary vitamin supplementation in due time, and improved nutrition in patients with Crohn's disease are quite important to prevent possible complications. Moreover, further studies with larger populations are needed to ascertain the risk factors that may bring about vitamin deficiencies in patients with CD.

Author contributions

Conceptualization: Sabiye Akbulut.
Data curation: Sabiye Akbulut.
Formal analysis: Sabiye Akbulut.
Investigation: Sabiye Akbulut.
Methodology: Sabiye Akbulut.
Validation: Sabiye Akbulut.
Writing – original draft: Sabiye Akbulut.
Writing – review & editing: Sabiye Akbulut.

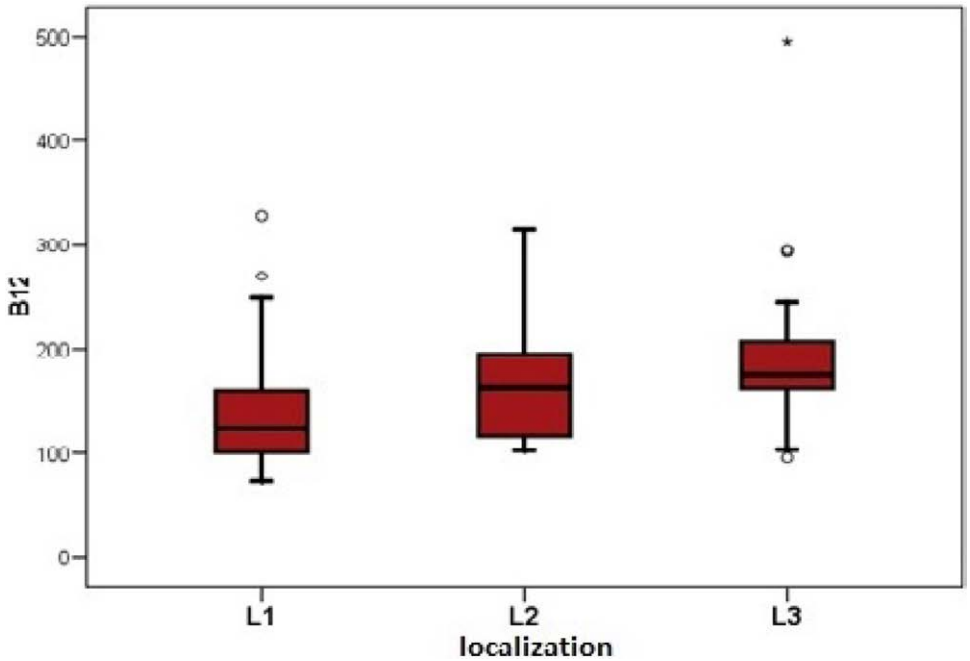

Figure 2. The minimum, maximum, 25–75% percentile, median B12 levels for the localization groups.

Table 4

B12 and FA pathology values for the patient and control groups.

	Patient group	Control group	
	n (%)	n (%)	*P*
Vitamin 12 < 126	37(35.9%)	3(2.6%)	<.001
Folic acid < 3	3(2.9%)	0(0.0%)	.105

References

[1] Lee SH, Kwon JE, Cho ML. Immunological pathogenesis of inflammatory bowel disease. Int Res. 2018;16:26–42.

[2] Agouridis AP, Elisaf M, Milionis HJ. An overview of lipid abnormalities in patients with inflammatory bowel disease. Ann Gastroenterol. 2011;24:181–7.

[3] Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002;347:417–29.

[4] Yu Y, Chen KC, Chen J. Exclusive enteral nutrition versus corticosteroids for treatment of pediatric Crohn's disease: a meta-analysis. World J Pediatr. 2019;15:26–36.

[5] Lees CW, Satsangi J. Genetics of infammatory bowel disease: implications for disease pathogenesis and natural history. Expert Rev Gastroenterol Hepatol. 2009;3:513–34.

[6] Aniwan S, Park SH, Loftus EV. Epidemiology, natural history, and risk stratification of Crohn's disease. Gastroenterol Clin N Am. 2017;46:463–80.

[7] Ao M, Tsuji H, Shide K, et al. High prevalence of vitamin B-12 insufficiency in patients. Asia Pac J Clin Nutr. 2017;26:1076–81.

[8] Sonawane K, Zhu Y, Chan W, et al. Association of serum folate levels with cardiovascular mortality among adults with rheumatoid arthritis. JAMA Netw Open. 2020;3:e200100.

[9] Pan Y, Liu Y, Guo H, et al. Associations between folate and Vitamin B12 levels and inflammatory bowel disease: a meta-analysis. Nutrients. 2017;9:382.

[10] Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a working party of the 2005 montreal world congress of gastroenterology. Can J Gastroenterol. 2005;19(Suppl A):5A5–36A.

[11] Levesque BG, Sandborn WJ, Ruel J, et al. Converging goals of treatment of inflammatory bowel disease from clinical trials and practice. Gastroenterology. 2015;148:37–51 e1.

[12] Cappellini MD, Motta I. Anemia in clinical practice-definition and classification: does hemoglobin change with aging? Semin Hematol. 2015;52:261–9.

[13] Gomollon F, Gargallo CJ, Munoz JF, et al. Oral cyanocobalamin is effective in the treatment of Vitamin B12 deficiency in Crohn's disease. Nutrients. 2017;9:E308.

[14] Allen LH, Miller JW, de Groot L, et al. Biomarkers of nutrition for development (BOND): Vitamin B-12 review. J Nutr. 2018;148:1995S–2027S.

[15] Balestrieri P, Ribolsi M, Pier M, et al. Nutritional aspects in inflammatory bowel diseases. Nutrients. 2020;12:372.

[16] Huang S, Ma J, Zhu M, Ran Z. Status of serum vitamin B_{12} and folate in patients with inflammatory bowel disease in China. Intest Res. 2017;15:103–8.

[17] Lamb CA, Kennedy NA, Raine T, et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. Gut. 2019;68:s1–s106.

[18] Altomare R, Damiano G, Abruzzo A, et al. Enteral nutrition support to treat malnutrition in inflammatory bowel disease. Nutrients. 2015;7:2125–33.

[19] Battat R, Kopylov U, Szilagyi A, et al. Vitamin B12 deficiency in inflammatory bowel disease: prevalence, risk factors, evaluation, and management. Inflamm Bowel Dis. 2014;20:1120–8.

[20] Yakut M, Ustün Y, Kabaçam G, et al. Serum vitamin B12 and folate status in patients with inflammatory bowel diseases. Eur J Intern Med. 2010;21:320–3.

[21] Lambert D, Benhayoun S, Adjalla C, et al. Crohn's disease and vitamin B12 metabolism. Dig Dis Sci. 1996;41:1417–22.

[22] Headstrom PD, Rulyak SJ, Lee SD. Prevalence of and risk factors for vitamin B12 deficiency in patients with Crohn's disease. Inflamm Bowel Dis. 2008;14:217–23.

[23] Park YE, Park SJ, Park JJ, et al. Incidence and risk factors of micronutrient deficiency in patients with IBD and intestinal Behçet's disease: folate, vitamin B12, 25-OH-vitamin D, and ferritin. BMC Gastroenterol. 2021;21:32.

[24] Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene. 2014;533:11–20.

[25] Mullin GE. Micronutrients and inflammatory bowel disease. Nutr Clin Ract. 2012;27:136–7.

[26] Somineni HK, Venkateswaran S, Kilaru V, et al. Blood-derived DNA methylation signatures of Crohn's disease and severity of intestinal inflammation. Gastroenterology. 2019;156:2254–2265.e3.

[27] Cario H, Smith DE, Blom H, et al. Dihydrofolate reductase deficiency due to a homozygous DHFR mutation causes megaloblastic anemia and cerebral folate deficiency leading to severe neurologic disease. Am J Hum Genet. 2011;88:226–31.

[28] Owczarek D, Rodacki T, Domagala-Rodacka R, et al. Diet and nutritional factors in inflammatory bowel diseases. World J Gastroenterol. 2016;22:895–905.
[29] Chowers Y, Sela BA, Holland R, et al. Increased levels of homocysteine in patients with Crohn's disease are related to folate levels. Am J Gastroenterol. 2000;95:3498–502.
[30] Olivera PA, Zuily S, Kotze PG, et.al. International consensus on the prevention of venous and arterial thrombotic events in patients with inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2021;18:1–17.
[31] Yazici A, Senturk O, Aygun C, et al. Thrombophilic risk factors in patients with inflammatory bowel disease. Gastroenterol Res. 2010;3:112–9.
[32] Harris D. Left middle cerebral artery infarct in a young man with Crohn's disease. J Royal Soc Med. 2009;102:443–4.
[33] Kallel L, Feki M, Sekri W, et al. Prevalence and risk factors of hyperhomocysteinemia in Tunisian patients with Crohn's disease. J Crohns Colitis. 2011;5:110–4.
[34] Liu EY, Loomes DE. Ustekinumab and vedolizumab dual biologic therapy in the treatment of Crohn's disease. Case Rep Med. 2017;2017:5264216.
[35] Caio G, Lungaro L, Caputo F, et al. Nutritional treatment in Crohn's disease. Nutrients. 2021;13:1628.
[36] Wiese D, Lashner B, Seidner D. Measurement of nutrition status in Crohn's disease patients receiving infliximab therapy. Nutr Clin Pract. 2008;23:551–6.
[37] Weiss G, Gasche C. Pathogenesis and treatment of anemia in inflammatory bowel disease. Haematologica. 2010;95:175–8.
[38] Gioxari A, Amerikanou C, Papada E, et al. Serum Vitamins D, B9 and B12 in Greek patients with inflammatory bowel diseases. Nutrients. 2020;12:3734.