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Factors associated with serum zinc levels in Iraqi patients with inactive inflammatory bowel disease

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ABSTRACT

Introduction: This study investigated relationship between dietary habits and serum zinc levels in patients with inflammatory bowel disease (IBD) during remission and identified factors contributing to zinc deficiency. In ulcerative colitis and Crohn's disease, low zinc levels can worsen gastrointestinal symptoms. However, current evidence regarding serum zinc levels in IBD patients remains conflicting. **Methods:** A retrospective cohort study was conducted at two hospitals, including 60 patients with IBD and 30 healthy controls. Participants completed a dietary questionnaire and their serum zinc levels were measured. **Results:** There were no significant differences in age (p=0.197) or sex (p=0.956) between groups. Employment and income levels were significantly lower in IBD patients (p<0.001 and p=0.004, respectively). Mean serum zinc levels were lower in IBD patients than controls (Crohn's: 71.2±8.2 µg/dL, ulcerative colitis (UC): 69.9±8.0 µg/dL vs. control: 91.9±20.7 µg/dL; p<0.001). Women had significantly lower zinc levels than men (73.6±10.8 vs. 82.1±20.9 µg/dL; p=0.016). Dietary intake of milk and legumes showed weak correlations with zinc levels (r=0.269, p=0.010; r=0.221, p=0.036). In the multivariate model, only being female (B=-11.273; p<0.001) and having IBD (Crohn's B = -18.26; UC B = -19.298; both p<0.001) remained significant predictors of lower zinc levels. **Conclusion:** Female sex and the presence of IBD were associated with lower serum zinc levels. Dietary intake did not significantly influence zinc levels. These findings suggest that monitoring zinc levels in IBD patients, particularly women, may be crucial for disease management.

Keywords: Crohn's disease, diet, ulcerative colitis, zinc

INTRODUCTION

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), affects an estimated 6.8 million people worldwide, with rising prevalence rates, particularly in newly industrialised countries (Jairath & Feagan, 2020). In the Middle East, the burden of IBD is increasing, posing a growing public health challenge (Alatab *et al.*, 2020).

Zinc deficiency has often been noticed in patients with chronic diarrhoea, malnutrition, and different catabolic states. Like copper, zinc deficiency is a common occurrence in IBD patients, both during the active phase of the disease and in remission (Kotze *et al.*, 2020). Zinc deficiency in UC may result from mucosal damage, malabsorption, and poor nutritional support attributed to active disease (Alatab *et al.*, 2020). In CD, low levels of zinc may further deteriorate GI symptoms, such as diarrhoea and gut motility, and provoke appetite and food choice disturbances. Besides, zinc

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deficiency delays intestinal healing and exacerbates the complications of the disease. In addition, growth retardation is also seen in young patients with low levels of zinc (Bonaventura *et al.*, 2014).

Zinc has a significant role in the maintenance of both the structural and functional integrity of the body (Cohen *et al.*, 2013). Zinc acts either as an activator or cofactor for many enzymes involved in growth, cell signalling, cellular functions, immune responses, and tissue repair (van Gennep *et al.*, 2021). Under conditions of intestinal inflammation and chronic malabsorption, deficiency in zinc probably results from micronutrient leakage starting at the beginning of the disease (Głąbska *et al.*, 2018). Reports indicate that even subclinical zinc deficits can induce mucosal inflammation, which may further aggravate colitis and enhance the production of proinflammatory cytokines.

Several factors can account for low zinc levels in individuals with IBD. These factors include malabsorption and possibly altered zinc transporters in the inflamed intestinal mucosa, enhanced losses due to inflammation and diarrhoea (Maywald & Rink, 2022), reduced dietary intake related to symptoms of IBD such as anorexia and dietary avoidance (Chow *et al.*, 2022; Kaliora, 2023; Yokokawa *et al.*, 2020), and oxidative stress, which impacts zinc metabolism and utilisation. The level of zinc in IBD patients remains debatable, with some studies reporting a normal level, while others showing a low serum zinc level (Casanova *et al.*, 2023).

This study aimed to investigate the relationship between serum zinc levels and a range of factors, including dietary habits, demographic characteristics, and clinical parameters, in Iraqi patients with IBD during the remission phase. The conceptual framework of this study is presented in Figure 1, illustrating how IBD, sociodemographic factors, and dietary habits interact to influence serum zinc levels among patients during the remission phase.

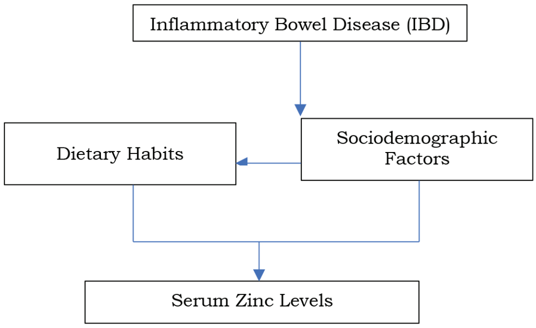

Figure 1. Conceptual framework illustrating the relationships between IBD, sociodemographic factors, dietary habits, and serum zinc levels among patients during remission

METHODOLOGY

This retrospective cohort study was conducted in two Iraqi hospitals, namely the Gastrointestinal Tract Teaching Hospital and the Baghdad Teaching Hospital, between July and September 2023. Ninety men and women, aged 18–56 years, were enrolled in this study. Sixty patients were in the remission phase from IBD (30 patients with CD and 30 patients with UC) and 30 healthy participants were matched as controls.

Patients were recruited by gastroenterologists at the hospitals via convenience sampling. The inclusion criteria were adults aged 18–56 years in the remission phase of IBD, with a confirmed diagnosis of either UC or CD. Healthy controls were selected from the same hospitals among hospital staff and patients' relatives and matched for age and sex. Exclusion criteria included other chronic diseases or conditions known to influence zinc metabolism. Blood sampling was performed by trained phlebotomists according to hospital procedures. Face-to-face interviews for food frequency questionnaire were conducted by trained research assistants to ensure accuracy and consistency. There was no payment or honorarium to the participants, but they were all informed of their zinc status at the end of the study.

Samples were obtained from healthy individuals and patients with IBD. Approximately 5 mL of blood was collected from each participant. Serum was isolated through centrifugation and stored at -40°C. Zinc was quantified using atomic absorption spectroscopy (novAA 300 by Analytik, Jena, Germany). Flame atomic absorption spectroscopy was employed for elemental analysis, utilising acetylene air as the flame source and hollow cathode lamps as the radiation source. A slit width of 0.7 nm was used to isolate the specific wavelength; absorbance was read at 213.9 nm.

A questionnaire was designed by the research team and completed through in-person interviews with the participants. The questionnaire assessed demographic information (e.g., age, sex), details regarding the disease (including type, disease duration, treatment duration, and remission period), socioeconomic factors (e.g., income, occupation), and dietary habits, (using the zinc-specific food frequency questionnaire).

The zinc-specific food frequency questionnaire consisted of 30 items, with ten focused on dietary intake. Food categories were selected based on their relevance to zinc bioavailability and dietary patterns commonly followed by IBD patients. These categories included protein sources (meat, chicken, fish, and eggs), dairy products (milk and cheese), legumes (lentils, chickpeas, and beans), grains and bread (wholegrain bread, rice), vegetables (leafy greens, carrots, cucumbers), fruits (apples, bananas, citrus fruits), nuts and seeds (almonds, walnuts, sunflower seeds), and processed foods (e.g., chocolate). These food items were assessed for frequency of weekly intake to explore potential dietary contributors to serum zinc levels. The questionnaire was adapted from validated tools and piloted among 20 participants for clarity and cultural appropriateness.

Data were imported from Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) to IBM SPSS Statistics for Windows version 26.0 (IBM Corporation, Armonk, NY, USA), where they were sorted and analysed. Categorical variables were presented as counts and percentages; their associations with the study groups were tested using Pearson's chi-square test or Fisher's exact test accordingly (if > 25% of the cells were <5). Numerical parameters were checked for normality using the Shapiro–Wilk test. Participants' age and zinc levels, which were found to be normally distributed, were presented as mean and standard deviation. The differences between study groups were tested using two-group or analysis of variance (>2 groups) parametric tests. All other numerical variables that were not normally distributed were presented as median and range. The differences according to study groups were tested using the Mann–Whitney U (two groups) or Kruskal–Wallis (>2 groups) non-parametric test. Spearman's non-parametric correlation was used to test the relationship between zinc levels and participants' age, years since diagnosis, remission, treatment, and weekly servings of food items. A multivariate linear regression model was used to assess the determinants of zinc levels among the study participants. The alpha level for significance was set at $p<0.05$. Multivariate linear regression analysis was conducted on the entire sample, including both IBD patients (CD and UC) and healthy controls ($n=90$). Study group classification (CD, UC, control) was included as an independent categorical variable in the model to

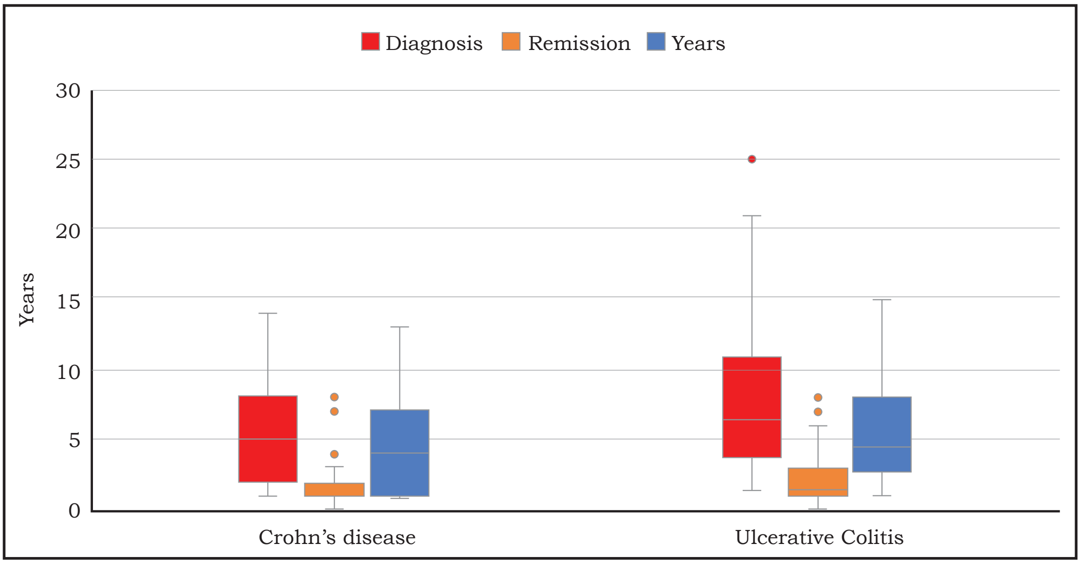

Figure 2. Comparison of years since diagnosis, remission, and treatment between patients with Crohn's disease and ulcerative colitis

assess its association with serum zinc levels alongside sociodemographic and dietary factors.

Before commencing this study, approval from the Supervising Committee of the Arab Board of Medical Specialisation was granted. In addition, the present study was approved by the Ethical Review Committee of the Iraqi Ministry of Health, with approval reference number MOH-IRB-2024-00123. The study was performed in compliance with ethical standards laid out in the Declaration of Helsinki. Participants were duly informed about the study; written informed consent was obtained from all participants before they took part in this study.

RESULTS

The results showed that approximately half of the study sample (47.8%) were unemployed (housewives, students, and job-seeking individuals), while 31.1% were employed. Two-thirds of the control group were employed, compared with 13.3% in each diseased group, indicating a significant association between occupation and IBD (p<0.001). The participants' income levels showed a significant difference across the study groups (p=0.004); notably, no individuals in the control group reported low income, whereas 20% of the UC group and 6.7% of the CD group fell into the low-income category (Table 1).

Regarding disease history, there were no significant differences between the CD and UC groups in terms of median years since diagnosis (p=0.051), years of remission (p=0.782), or years of treatment (p=0.302). Overall, the median duration since diagnosis among IBD patients was 5.5 years, ranging from 1 to 25 years. Patients with UC had a slightly longer median disease duration of 6.5 years (range 1.5–25.0 years) compared to 5 years (range 1–14 years) in those with CD.

The median remission period for all IBD patients was 1 year (range: 0–8 years). Specifically, UC patients had a longer remission duration (median 1.5 years; range 1 month to 8 years) compared to CD patients (median 1 year; range 1 month to 8 years), although

Table 1. General characteristics and weekly dietary intake across study groups

Variable	*CD (n=30)*	*UC (n=30)*	*Control (n=30)*	*Total (n=90)*	*p-value*
Age (years) (Mean±*SD*)	29.2±8.9	32.2±9.8	33.5±9.7	31.6±9.5	0.197†
Sex (%)					0.956
Male	50.0	46.7	46.7	47.8	
Female	50.0	53.3	53.3	52.2	
Education level (%)					0.776
Primary or less	6.7	13.3	10.0	10.0	
Intermediate/Secondary	56.7	43.3	43.3	47.8	
College or higher	36.7	43.3	46.7	42.2	
Marital status (%)					0.870
Never married	43.3	36.7	40.0	40.0	
Ever married	56.7	63.3	60.0	60.0	
Occupation (%)					<0.001***
Unemployed	46.7	63.3	33.3	47.8	
Self-employed	40.0	23.3	0.0	21.1	
Employed	13.3	13.3	66.7	31.1	
Income (%)					0.004**
Low	6.7	20.0	0.0	8.9	
Medium	83.3	56.7	96.7	78.9	
Good	10.0	23.3	3.3	12.2	
Weekly food servings (Median, Range)					
Meat	2 (0–35)	2 (0–21)	2 (0–14)	2 (0–35)	0.833
Chicken	3 (0–70)	4 (0–7)	3 (0–7)	3 (0–70)	0.412
Fish	1 (0–5)	1 (0–2)	1 (0–3)	1 (0–5)	0.789
Egg	7 (0–35)	6 (0–28)	4 (0–14)	6 (0–35)	0.024*
Milk	3 (0–14)	3 (0–14)	6 (0–10)	3 (0–14)	0.202
Cheese	2 (0–21)	3 (0–21)	5 (0–14)	3 (0–21)	0.175
Bread	42 (0–168)	28 (1–112)	42 (0–84)	42 (0–168)	0.878
Rice	14 (0–56)	14 (0–28)	14 (0–84)	14 (0–84)	0.280
Vegetables	14 (0–21)	7 (0–21)	14 (3–28)	14 (0–28)	0.185
Fruit	7 (0–21)	7 (0–28)	14 (0–21)	7 (0–28)	0.486
Legumes	2 (0–7)	1 (0–3)	2 (0–6)	2 (0–7)	0.002**

CD: Crohn's disease; UC: ulcerative colitis; SD: Standard deviation
†ANOVA, Kruskal-Wallis for non-parametric
*Significant at p<0.05; **Significant at p<0.01; ***Significant at p<0.001

one CD patient had not yet achieved remission at the time of the study.

The overall median years of treatment were 4.5 years and ranged from 10 months to 15 years. The median years of treatment in the CD group were 4 years (ranging from 10 months to 13 years), while it was 4.5 years and ranged from 1 to 15 years in the UC group (Figure 2).

Participants were asked about each food item they usually consumed and to provide details about their weekly consumption. Results showed that the weekly median servings for egg consumption in the control group (4, range: 0–14) was significantly lower (p=0.024) than that in the diseased groups [CD (7, range: 0–35) and UC (6,

Table 2. Serum zinc levels by demographics, disease status, and dietary correlates

Variable	*Zinc level*		*p-value*
	Mean±SD	*r*	
Sex			0.016*
Men	82.1±20.9		
Women	73.6±10.8		
Education level			0.885
Primary or less	79.3±18.6		
Intermediate/Secondary school	76.8±15.8		
College or higher	78.2±18.0		
Marital status			0.545
Never married	76.4±14.7		
Ever married	78.5±18.3		
Occupation			0.001**
Unemployed	76.9±12.5		
Self-employed	67.8±8.5		
Employed	85.4±22.7		
Income			0.024*
Low	66.4±6.0		
Medium	80.1±17.7		
Good	70.0±10.0		
Type of disease			<0.001***
Crohn's disease	71.2±8.2		
Ulcerative colitis	69.9±8.0		
Control	91.9±20.7		
Correlations with zinc level			
Age		0.083	NS
Years since diagnosis		0.093	NS
Years of remission		-0.086	NS
Years of treatment		0.212	NS
Weekly meat servings		0.197	NS
Weekly milk servings		0.269	0.010*
Weekly legume servings		0.221	0.036*

NS: Not significant
*$p<0.05$; **$p<0.01$; ***$p<0.001$

range: 0–28), respectively]. The weekly median number of servings for legumes was significantly lower ($p = 0.002$) in the UC group (1, range: 0–2) compared with the CD (2, range: 0–7) and control groups (2, range: 0–6). None of the other food items showed significant differences in median weekly consumption across the study groups (Table 1).

The average zinc level of the participants was 77.6±16.9 µg/dL. Mean zinc level in women was significantly lower than in men (73.6 µg/dL vs. 82.1 µg/dL, p=0.016). Zinc level also showed a significant difference based on participants' occupation, at 67.8±8.5 µg/dL among self-employed participants, which was significantly lower (p=0.001) than that in unemployed (76.9±12.5 µg/dL) and employed participants (85.4±22.7 µg/dL). Zinc level in the control group was 91.9±20.7 µg/dL, which was significantly higher (p<0.001) than that in the CD (71.2±8.2 µg/

Table 3. Multivariate linear regression on factors predicting serum zinc levels

Variable	*B Coefficient*	*95% CI (Lower–Upper)*	*p-value*
Constant	105.829	95.249 – 116.409	<0.001***
Sex			
Women (vs. men)	-11.273	-17.046 – -5.500	<0.001***
Occupation (vs. employed)			
Unemployed	+2.839	-4.458 – +10.137	0.441
Self-employed	-5.609	-14.963 – +3.745	0.236
Study group (vs. control)			
Crohn's disease	-18.260	-26.268 – -10.252	<0.001***
Ulcerative colitis	-19.298	-27.742 – -10.853	<0.001***
Income (vs. medium)			
Low income	-5.861	-16.085 – +4.364	0.257
Good income	-1.296	-10.15 – +7.559	0.772
Dietary servings (per week)			
Milk	+0.409	-0.351 – +1.168	0.287
Legumes	+0.240	-1.682 – +2.161	0.804

R^2=0.354; Adjusted R^2=0.312; Model *p*-value<0.001 (Good fit) Multivariable linear regression analysis showed that diagnosis of IBD and sex (female) were independent predictors of serum zinc. Serum zinc levels in women were significantly lower compared to men (*B*=-11.273, 95% CI: -17.046 to -5.5, p<0.001). In the same way, Crohn's disease patients (*B*=-18.260, 95% CI: -26.268 to -10.252, p<0.001) and ulcerative colitis patients (*B*=-19.298, 95% CI: -27.742 to -10.853, p<0.001) were significantly lower in zinc compared with healthy controls. Status of occupation, income, weekly milk servings, and weekly legume servings were also added as covariates but were not significantly associated with zinc levels (p>0.05). The model accounted for 35.4% of variance in zinc concentration (R^2=0.354, adjusted R^2=0.312) with overall model *p*-value <0.001, showing a good fit. ***p<0.001

dL) and UC (69.9±8.0 µg/dL) groups, respectively. However, neither education level nor marital status showed a significant effect on zinc levels (Table 2).

Zinc levels of participants were also tested for correlations with age, years since diagnosis, and years of remission and treatment, with no significant correlations. In addition, zinc levels of participants appeared to be significantly correlated with weekly servings of milk (r=0.269, p=0.010); however, this correlation was weak, with milk servings affecting zinc level by 7% (r^2=0.072). A similar correlation was found between zinc level and weekly servings of legumes (r=0.221, p=0.036); the effect of legume consumption on the zinc level of participants was approximately 5% (r^2=0.049). No other food items significantly correlated with zinc levels (Table 3).

Previous analysis showed that zinc levels had more than a significant relationship with participants' sex, occupation, income, and weekly servings of milk and legumes, and there were significant differences between study groups. Thus, further analysis was used to adjust those variables with each other to eliminate confounders.

The regression model identified two determinants that interacted with zinc level significantly – being a woman and having any type of IBD, both tremendously decreased zinc levels. Zinc levels in women were significantly lower than those in men [*B* = -11.273, 95% confidence interval (*CI*): -17.046 to -5.5] and in patients with CD (*B* = -18.26, 95% CI: -26.268 to -10.252) and UC (*B* = -18.26, 95% *CI*: -27.742 to -10.853) compared to those in the control group. All other variables appeared to be confounders (Table 3).

DISCUSSION

IBD is a chronic relapsing-remitting immunological condition of unknown cause where nutritional therapy is regarded as the supportive or primary treatment (Poursadegh, Hassanzadeh & Khani, 2018). Our study reported a significant association between occupation and IBD in two-thirds of the control group, whereas only 13.3% of the diseased group were employed. This result is consistent with previous studies (Alatab *et al.*, 2020). This might be explained by the effect of IBD on productivity, as IBD may negatively influence work productivity and lead to higher unemployment and work disability rates than in healthy populations (van Gennep *et al.*, 2021). In addition to the association with occupation, IBD was also significantly associated with low income, in line with previous studies (Alatab *et al.*, 2020) too. Once again, this might be explained by the effect of IBD on work productivity.

The weekly median consumption of eggs was significantly higher in patients with IBD than in the control group. This might be because the Mediterranean diet is usually recommended for people with IBD, which emphasises protein intake mainly from fermented dairy products, eggs, and white meat (Ratajczak *et al.*, 2023; Radziszewska, Brzozowski & Magierowski, 2022). The weekly median number of servings for legumes was significantly lower in the UC group than that in the CD and control groups. However, previous studies have reported decreased legume intake in both UC and CD patients, as patients believe that legume intake may exacerbate disease symptoms.

The mean serum zinc level in the CD group was 71.2±8.2 μg/dL and 69.9±8.0 μg/dL in the UC group, similar to previous studies showing that the median zinc level in patients with CD was 74.3±9.7 μg/dL (Sakurai *et al.*, 2022) and 72.6±15.2 μg/dL in those with UC (Shokrzadeh *et al* 2013). Zinc level in the control group of this study was 91.9±20.7 μg/dL, which is in line with the Japanese Society of Clinical Nutrition's indication of a normal serum zinc level as ≥80 μg/dL (Yokokawa *et al.*, 2020).

After adjustment in the regression model, serum zinc level had only two determinants: being a woman and having any type of IBD. These two factors have been shown to significantly decrease serum zinc levels. Lower serum zinc levels have been previously reported in women (Ruangritchankul *et al.*, 2023). This difference in zinc levels between sexes could be because of the difference in muscle mass, as approximately 60% of zinc in the body is present in muscles (Chow *et al.*, 2022). Previous studies have shown that serum zinc levels are higher in men with larger muscle mass (Chow *et al.*, 2022). In our study, zinc levels in patients with CD and UC were significantly lower than those in the control group. Previous studies reported low serum zinc levels in patients with IBD (Zupo *et al.*, 2022; Soltani *et al.*, 2021). This result could be because the zinc transporter albumin might be affected by low albumin levels, which is more common in patients with IBD who are malnourished or have malabsorption, a higher fractional catabolic rate of albumin, and higher albumin transfer out of the vascular system (Wessels *et al.*, 2022).

After adjustment in the regression model, serum zinc levels were found to be unaffected by food consumption. Ruangritchankul *et al.* (2023) showed no relationship between dietary zinc intake and serum zinc levels. Wessels *et al.* (2022) reported no association between dietary intake and serum zinc concentration. Their meta-analysis reported that zinc deficiency in patients with IBD may result from poor oral intake, especially because of the intrinsic malabsorptive nature of IBD. Besides that, they also found that low serum albumin and depression were the main predictors of low serum zinc levels rather than low dietary intake (Wessels *et al.*, 2022).

Study limitations

This study has limitations, including the relatively small sample size, its cross-sectional design, and reliance on self-reported dietary data, which may introduce recall bias. Additionally, the study was conducted in two hospitals, limiting its generalisability to the broader Iraqi population.

Recommendations

Based on the findings of this study, it is recommended that routine nutritional screening protocols be developed to monitor serum zinc levels in patients with IBD, particularly focusing on female patients and those diagnosed with CD or UC, even during periods of clinical remission.

Additionally, future intervention studies are needed to assess the effectiveness of targeted dietary modifications or zinc supplementation strategies in improving serum zinc levels among IBD patients. Research should also explore whether early nutritional interventions could contribute to better disease management and improved quality of life in this population.

CONCLUSION

Although patients with IBD maintained clinical remission, their serum zinc levels were lower than the control group. The determinants of low serum zinc levels were being a woman and having any type of IBD. Dietary intake and food consumption were not determinants of serum zinc levels. Despite maintaining clinical remission, IBD patients should undergo systematic laboratory tests for assessing micronutrient deficiencies, including serum zinc levels. Further studies on the risk factors associated with low serum zinc levels are recommended. As patients with IBD experience reduced productivity, gastroenterologists should be more aware of the impact of IBD on patients' work lives and raise the issue with patients to guide them with individually tailored action plans.

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Authors' contributions

Ruaa EA, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript, and reviewed the manuscript; Hayder GO, advised on data analysis and interpretation, and reviewed the manuscript; Nawal Mehdi FA, led the data collection and clinical assessments, contributed to data interpretation, and reviewed the manuscript.

Conflict of interest

The authors have no competing interests to declare.

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