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Biochemical Markers of Iron Status Among Iraqi Patients at Different Severity Levels of Celiac Disease

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Abstract

In people with a hereditary predisposition, gluten consumption causes celiac disease, a multifactorial autoimmune condition. The ensuing small intestine inflammatory process develops specific antibodies and causes various gastrointestinal and extra-intestinal symptoms with varying degrees of severity. Evaluation of the blood iron profile at various histological celiac disease severity in Iraqi patients was the goal of the current study. Seventy-five Iraqi patients with celiac disease, with a mean age of 18.68± 11.13 years, were the study's participants. These people tested positive for celiac antibodies and experienced gastrointestinal symptoms. Based on the disease histological severity, they were divided into two groups: marsh (III a, b, c) group and marsh (0, I) group. For comparison purposes, 46 people who appeared to be in good health and matched in age and gender to the patients utilized in the study were also included. The levels of iron, transferrin, hemoglobin, ferritin, total iron binding capacity, unsaturated iron binding capacity, and the percentage of transferrin that is saturated with iron were all measured. In comparison to the other patient groups under study, celiac patients (marsh III) group, and particularly those of group (marsh III c), showed a substantial drop ($p<0.05$) in iron, the percentage of transferrin saturating with iron, the levels of each ferritin and of hemoglobin. The total iron binding capacity and the unsaturated iron binding capacity showed a significant elevation ($p<0.05$) in (marsh III c) patients' group, meanwhile non-significant differences ($p> 0.05$) were found in transferrin concentration. Moreover, significant differences ($p< 0.05$) were obtained in the iron profile between celiac patients who followed strict gluten free diet and those patients with gluten contained diet.

Keywords: ferritin, transferrin, iron, mucosal histopathological damage, anemia, gluten free diet, gluten containing diet

واسمات كيموحيوية لحالة الحديد عند المرضى العراقيين ذوي المستويات المختلفة من مرض حساسية الحنطة

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الخلاصة

مرض حساسية الحنطة وما يعرف ايضا باعتلال الامعاء الحساس للكلوتين هو اضطراب مناعي. هدف الدراسة الحالية كان تقييم نمط الحديد في مصل الدم للمرضى العراقيين المصابين بحساسية الحنطة خلال مراحل مختلفة الشدة من امراضية النسيج. كان عدد المشاركين في الدراسة خمس وسبعين مريض عراقي في مدى عمري (18.11 ± 13.11) سنة. كان لدى هؤلاء المرضى اعراض معدية معوية ونتيجة موجبة للاجسام المضادة الخاصة بمرض حساسية الحنطة. وقسم المرضى بناء على شدة امراضية الانسجة الى مجموعتين : مجموعة marsh (III a, b, c) ومجموعة marsh (0,I). شملت الدراسة ايضا لاغراض المقارنة ستة و اربعين شخصا اصحاء ظاهريا ومتوافقين بالعمر والجنس مع المرضى المستخدمين في الدراسة. وقد تم خلال الدراسة قياس تركيزكل من الحديد والترانسفيرين والهيموغلوبين وكذلك الفريتين وقابلية ارتباط الحديد الكلية وقابلية عدم تشبع الحديد والنسبة المئوية لتشبع الترانسفرين بالحديد.

وجد ان مرضى مجموعة (marsh III) وخاصة اولئك الذين لديهم ضمور في الزغابات (marsh III c) يعانون نقوصات ذات دلالة احصائية ($p < 0.05$) في الحديد وفي النسبة المئوية لتشبع الترانسفرين بالحديد وفي مستويات كل من الفريتين والهيموغلوبين مقارنة ببقية المحاميع المدروسة. اظهرت قدرة ارتباط الحديد الكلية و قدرة عدم تشبع ارتباط الحديد ارتفـاع ذات دلالة احصائية ($p <0.05$) في المرضى مجموعة (marsh III c) وفي نفس الوقت وجدت فروقات غير محسوسة ($p>0.05$) في تركيز الترانسفرين. اضافة الى ذلك حصلنا على فروقات محسوسة ($p < 0.05$) في نمط الحديد بين مرضى حساسية الحنطة الذين يتناولون طعام خالي تماما من الكلوتين واولئك الذين يتناولون طعام يحتوي الكلوتين .

Introduction

It is well recognized that dietary gluten is the primary initiator of celiac disease (CD), a chronic immune-mediated enteropathy [1]. The term "gluten" is a catch-all term for proteins that are soluble in alcohol [2], which is thought to be the main storage protein in wheat [3]. In response to gluten exposure, the small intestinal mucosa had distinctive histologic abnormalities, such as lymphoplasmacytic infiltration of the lamina propria and intraepithelial lymphocytosis with various degrees of villous atrophy [4]. Demographic studies show that this widespread, worldwide illness is becoming more prevalent in many nations [5]. The genetic and environmental variables that contribute to CD pathogenesis are complicated [6]. This disease affecting approximately 1% of the population [1] and reported to occur in all age groups [7].

A common laboratory result in CD patients is anemia and/or iron deficiency [8]. According to several studies, anemia affects around (12-69%) of those who have just received a CD diagnosis [9]. The site of most documented damage in CD patients has been shown to be the proximal small intestine, which is also where iron is often absorbed. The binding of iron and other metals in the blood to various transport proteins, including hemoglobin, transferrin, and ceruloplasmin, as well as to intracellular storage proteins such as ferritin, sequesters them from interaction with reactive oxygen species (ROS) and O_2 in the body in a nontoxic and easily accessible form in the cell [10, 11].

This study aimed to determine the occurrence of iron deficiency anemia (IDA) in a sample of celiac patients while concentrating on iron status in connection to various histological levels of disease severity and gluten-free diet (GFD).

Materials and Methods

Seventy-five patients in total with several key characteristics, such as bloating, chronic stomach discomfort, chronic diarrhea, or if they have a positive CD-antibody, were included in this study. They were present in the Gastroenterology and Hepatology Center in Baghdad. Their

ages varied from 2 to 43 years, and they were requested to complete a specially created questionnaire with all relevant details, including age, gender, symptoms, the presence or absence of gluten in their diet, and any autoimmune disorders they may have had.

Forty-six participants who served as the control group were registered patients who appeared to be in good health. They had no history of any illnesses, particularly those that were gastrointestinal in nature.

In addition to further testing of the stomach, esophagus, and duodenum, at least three biopsies were collected from various locations in the distal region of the duodenum. The histological examinations of the biopsies, which were put in 10% formalin in a ground glass tube, were carried out by two blinded expert pathologists [12].

The diagnosis of the disease was based on increased intraepithelial lymphocyte counts with the presence of total, subtotal or partial villous atrophy on initial endoscopic biopsies. Marsh 1992 classification was followed in the histological analyses in which: partial villous atrophy was scored as Marsh III a, subtotal villous atrophy as Marsh III b, and total villous atrophy as Marsh III c [13, 14]

The following biochemical parameters were determined**:** (Hb) using Drabkin's Reagent [15], serum iron [16], transferrin [17]**,** and serum ferritin was measured using Randox Kit (Randox Laboratories/ United Kingdom), which based on radioimmunoassay method and the enzyme linked fluorescent assay technique (ELFA) in which the assay principle combines a one-step immunoassay sandwich method with a final fluorescent detection.

Statistical Analysis

The data expressed as mean value± SD, which were analyzed using the SPSS software Meantime t- test was used for differences' analysis between the studied groups. ($p < 0.05$) was considered as statistically significant, while ($p \leq 0.001$) as a highly significant. Each of the analysis were repeated (3) times.

Results & Discussion

Table 1 illustrates the results of iron profile represented by [iron], [transferrin], total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), saturation % of transferrin, concentrations of ferritin and Hb in sera of the patients at different histopathological severity of the disease. The results in the same table showed that, in comparison to the healthy control group, the patients who had substantial histopathological damage had considerable elevation in TIBC ($p=0.019$) and extremely significant elevation in UIBC (p=0.000). The measured amounts of iron and hemoglobin (Hb) were significantly decreased in conjunction with these changes ($p=0.001$ and $p=0.036$ respectively). Comparing patient groups to healthy participants, a very significant decrease ($p=0.000$) was noted in the transferrin saturation % and ferritin concentrations, although a non-significant change ($p>0.05$) was noted in the transferrin concentration.

Table 1: Characteristic of iron profile and hemoglobin in sera of control and patients with celiac disease

	Groups			
	Control	Patients Marsh III a, b, c	Patients Marsh 0,I	
	Mean value± SD (range)			
Age (years)	15.805±10.324 (4-40)	14.58±9.772 (2-43)	17.807±11.707 (4.5-38)	Non sig. *p > 0.05*
[Iron] µg/dL	92±28.3 (65-156)	55.66±21.48 (27-101)	84±37.2 (55- 153)	Sig ↓ for patients Marsh III *p= 0.001*
TIBC µg/dL	321.66±38.78 (269-412)	349.12±44.19 (283-453)	304±54,24 (229-403)	Sig.↑ for patients Marsh III *p= 0.019*
UIBC µg/dL	230.14±30.18 (204-256)	293.46±33.73 (265-352)	219.57±34.06 (174-250)	Sig. ↑ for patients Marsh III *p= 0.000*
[Transferrin] mg/dL	225.91±31.88 (188-285)	244.38±55.7 (198-317)	210±39.11 (156-280)	Non sig. *p> 0.05*
%Saturation of transferrin	28.69±9.56 (24.1-38.04)	16.14±05.3 (9.34-23.28)	27.41±8.63 (23.2-37.9)	Sig.↓ for patients Marsh III *p= 0.000*
[Ferritin] ng/ml	152.67±69.77 (19-300)	103.89±54.56 (10-253)	146.45±61.12 (23-281)	Sig. ↓ for patients Marsh III *p= 0.000*
Hb g/dL	13.82 ± 2.76 (10-17)	*9.64 ± 3.20 (6.5-16)	12.36±2.43 (9-18)	Sig. ↓ *p= 0.036*

* The difference is significant at ($P \leq 0.05$).

According to the findings in Table 2, severe celiac disease patients (Marsh III c) had substantial increases (*p=0.005* and *p=0.021*) in both TIBC and UIBC, as well as significant decreases in iron, ferritin, and the saturation percentage of transferrin. Nevertheless, celiac patients from Marsh III a and III c had significantly elevated transferrin levels (*p=0.027*). Ultimately, in category III c and b celiac patients, the Hb level significantly decreased (*p=0.000*) and (*p=0.003*).

Table 2: Mean values ± SD of the iron profile and hemoglobin level in sera of control and (Marsh III a, b, c) severe histopathological damage patients.

	Groups				*P value*
	Control	Patients at Marsh III a	Patients at Marsh III b	Patients at Marsh III c	
	Mean value± SD (range)				
Age (years)	15.805±10.324 (4-40)	13.956±9.479 (2-43)	14.144±9.565 (2.5-33)	19.400±12.130 (5-39)	Non sig. *p> 0.05*
[Iron] µg/dL	92±28.3 (65-156)	59.31±21.23 (33-95)	61.93±25.61 (31-101)	47.11±16.12 (27- 67)	Sig.↓ *p= 0.000*
TIBC µg/dL	321.66±38.78 (269-412)	346.74±40.2 (285-450)	334.39±51.1 (283-434)	363.68±32,3 (287-453)	Sig.↑ *p= 0.005*
UIBC µg/dL	230.14±30.18 (204-256)	286.73±30.12 (252-355)	273.08±42.4 (252-333)	316.18±29.56 (260-386)	Sig.↑ *p=0.021*
[Transferrin] mg/dL	225.91±31.88 (188-285)	242.02±39.29 (199-315)	233.8±44.83 (198-303)	254.1±30.05 (200-317)	Sig.↑ *p=0.027* for patients Marsh III a & c
%Saturation of transferrin	28.69±9.56 (24.1-38.04)	17.25±06.52 (11.57-21.11)	18.54±8.37 (10.95-23.28)	12.83±4.19 (9.34-14.69)	Sig.↓ *p=0.003*
[Ferritin] ng/ml	152.67±69.77 (19-300)	121.89±60.50 (77-253)	88.77±51.43 (18-252)	77.42±36.9 (10-200)	Sig.↓ *p=0.014*
Hb g/dL	13.82 ± 2.76 (10-16)	11.55± 1.83 (8-15)	**9.1± 3.23 (7-16)	*8.37± 1.83 (6.5-15)	*Sig. ↓ *P= 0.000* ** Sig. ↓ *P= 0.003*

*The difference is significant at ($P \leq 0.05$).

The levels of blood TIBC, UIBC, and transferrin in the male's marsh III celiac patient group revealed a significant rise when iron profile was assessed independently in male and female study groups as shown in Table 3 ($p < 0.05$), and the similar results were observed for the women patients within the same group. While there were significant differences between the men and women with stage III celiac disease and the healthy men and women ($p < 0.05$) in terms of iron levels, serum transferrin saturation percentage, ferritin levels, and hemoglobin concentration.

Table 3: The mean values ± SD of iron profile and Hb in the sera of both control and patients with celiac disease according to gender distributions

		Groups			P value
		Control (♂=20 ,♀=26)	Patients Marsh III a, b, c (♂=24 ,♀=38)	Patients Marsh 0,I (♂=7,♀=6)	
	Mean value± SD (range)				
Age (years)	♂	16.84±9.654 (6-38)	14.217±10.621 (2-39)	17.071±14.289 (4.5-38)	
	♀	14.64±10.997 (4-40)	15.084±10.039 (2-43)	18.666±9.07 (2-38)	**Non sig.**
P value		***0.474***	***1.000***	***0.778***	
[Iron] µg/dL	♂	107.94±29.0 (80-156)	57.65±23.65 (27-101)	89.87±31.3 (80- 153)	
	♀	81.58±19.3 (65-149)	53.87±20.15 (27-70)	79.45±39.52 (55- 151)	Sig.↓ *p= 0.000* for patients Marsh III
P value		***0.000***	***0.087***	***0.000***	
TIBC µg/dL	♂	320.10±37.17 (269-410)	349.11±44.67 (289-453)	310.43±56,19 (229-403)	**Sig.**↑ *p=0.0048* for patients Marsh III
	♀	323.22±39.93 (294-412)	348.00±43.11 (283-449)	300.05±53,25 (237-403)	
P value		***0.089***	***0.476***	***0.018***	
UIBC µg/dL	♂	213.17±23.63 (189-255)	293.65±34.50 (262-352)	221.04±36.61 (179-250)	**Sig.**↑ *p=0.003* for patients of Marsh III.
	♀	243.45±41.54 (228-256)	296.08±40.36 (256-379)	222.80±26.01 (174-239)	
P value		***0.000***	***0.085***	***0.538***	
[Transferrin] mg/dL	♂	225.97±32.08 (188-281)	244.28±50.7 (202-317)	217±39.55 (156-271)	**Sig.**↑ *p=0.04* for patients Marsh III
	♀	226.51±30.00 (209-285)	243.05±56.02 (198-315)	211±36.10 (160-280)	
P value		***0.480***	***0.330***	***0.078***	
%Saturation of transferrin	♂	33.35±11.11 (29.78-38.04)	16.50±5.10 (9.34-23.88)	28.72±8.65 (25.84-37.9)	**Sig.**↓ *p=0.000* for patients Marsh III
	♀	25.00±8.87 (22.1-36.1)	15.51±5.56 (9.5-15.62)	26.45±8.92 (23.2-37.5)	
P value		***0.003***	***0.215***	***0.079***	
[Ferritin] ng/ml	♂	177.56±79.77 (40-300)	120.13±55.31 (29-253)	148.91±64.22 (30-281)	**Sig.**↓for bo patients 'gro *P=0,0* for ♂
	♀	139.67±58.9 (19-279)	88.89±52.11 (10-240)	143.32±57.54 (23-273)	**Sig.**↓ *p=0.01*for♀ patients Mars III
P value		***0.001***	***0.000***	***0.335***	
Hb g/dL	♂	15.07±1.74 (14-16)	*11.00±2.82 (9-16)	14.19±2.93 (10-18)	***Sig.**↓ *P= 0.01*
	♀	12.83±3.9 (10-15)	**8.51±3.00 (6.5-15)	11.02±2.58 (9-16)	****Sig.**↓ *P= 0.043*
P value		***0.027***	***0.04***	***0.055***	

*The difference is significant at ($P \leq 0.05$).

According to results in Table 3, there were significant differences between the healthy male and female control groups for serum iron (*p=0.000*), UIBC (*p=0.000*), transferrin saturation percentage (*p=0.003*), ferritin levels (*p=0.001*), and Hb concentration (*p=0.027*) when the comparison was made based on gender distribution. Additionally, there were significant differences between the male and female marsh 0, I celiac patient groups for serum iron (*p=0.000*) and TIBC (*p=0.018*), while the male and female marsh III celiac patient groups showed highly significant differences (*p=0.000*) and significant differences (*p=0.04*, respectively) for serum ferritin level and hemoglobin concentration.

The relationship between diet and serum iron profile is presented in Table 4. The group of CD patients on gluten contain diet were distributed around a much lower mean value for iron (48.32±16.3 µg/dL), transferrin saturation percentage (13.76±05.1) and ferritin (99.49±44.27 ng/ml) in comparison to the healthy control group. While significant elevation was observed in the mean values of TIBC (351.31±39.11µg/dL) and UIBC (303.1±33.73 µg/dL) for the same patients group.

Table 4: Mean values ± SD of iron profile and [hemoglobin] in the sera of celiac Iraqi patients, with, or free gluten diet & the control group.

	Groups			*P value*
	Control (n=46)	Patients on gluten diet (n=55)	Patients on gluten free diet (n=20)	
	Mean value± SD (range)			
Age (years) Mean± SD (Range)	15.805±10.324 (4-40)	14.14±10.14 (2-43)	15.69±9734 (3-33)	**Non sig.**
[Iron] µg/dL	92±28.3 (65-156)	48.32±16.3 (27-101)	89±29.77 (59- 153)	**Sig.↓** for GCD gp *P= 0.000*
TIBC µg/dL	321.66±38.78 (269-412)	351.31±39.11 (283-453)	309±43,92 (232-403)	**Sig.↑** for GCD gp *P= 0.001*
UIBC µg/dL	230.14±30.18 (204-256)	303.1±33.73 (256-352)	220±34.06 (173-250)	**Sig. ↑** for GCD gp *P= 0.000*
[Transferrin] mg/dL	225.91±31.88 (188-285)	245.91±47.8 (198-318)	216.3±39.11 (162-280)	**Non sig**.
%Saturation of transferrin	28.69±9.56 (24.1-38.04)	13.76±05.1 (9.54-22.62)	28.86±8.53 (25.6-37.9)	**Sig↓** for GCD gp. *P= 0.001*
[Ferritin] ng/ml	152.67±69.77 (19-300)	99.49±44.27 (10-241)	151.93±61.17 (23-313)	**Sig. ↓** for GCD gp *P= 0.005*.
Hb g/dL	13.82 ± 2.76 (10-17)	10.33 ± 3.08 (6.5-18)	12.36±2.84 (8-18)	**Non sig**.

** GCD= gluten contain diet

The IDA was found in 17 cases (27.4%) patients with partial, subtotal, and total villous atrophy celiac group (Marsh III a, b, ,c) (5 male and 12 female), and (2.3%) patients with less villous atrophy celiac group (Marsh 0, I) compared with control group (0%) as shown in Figure 1. The ratio of IDA in celiac males and females patients was also evaluated as shown in Figure 2.

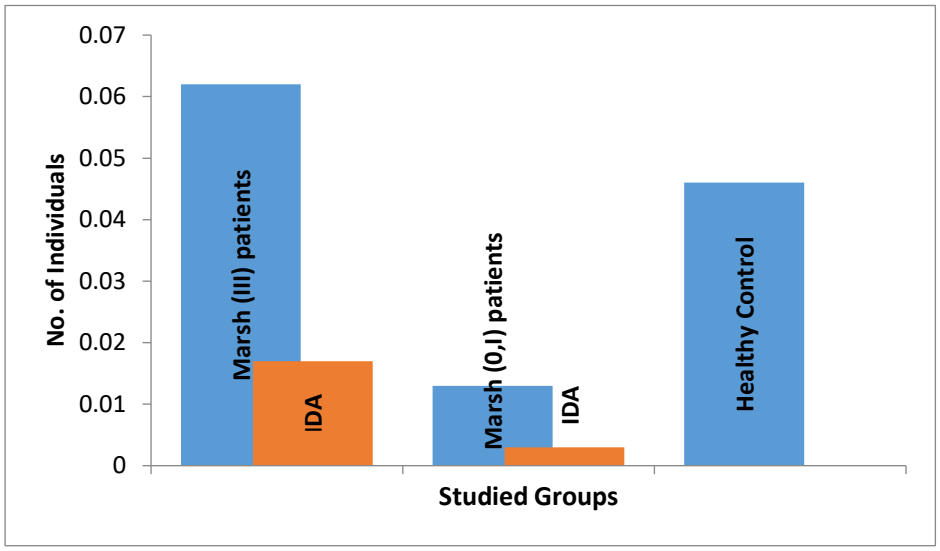

Figure 1: The levels of Iron deficiency anemia in all studied groups

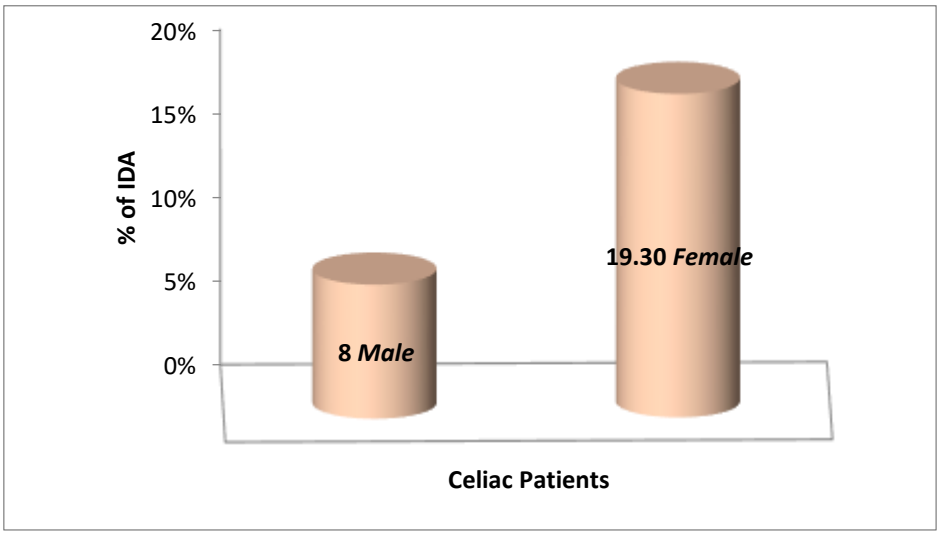

Figure 2: The levels of Iron deficiency anemia in celiac patients group according to gender

The clinical diagnosis of ID, as well as a screening test for other clinical problems [18], are thought to benefit from the measurement of the iron level, TIBC, and percentage of the transferrin saturation with iron [17]. Moreover, determining plasma TIBC is a common way to measure plasma transferrin [19].

The quantity of iron was previously assessed in a number of CD-related investigations, and iron insufficiency was found to be the most common nutritional shortfall caused by decreased absorption [20]. This was supported by the findings of the current study, which showed that patients with Marsh III a, b, and c, and particularly those with Marsh III c, had statistically lower iron levels than patients with Marsh 0, I, or patients with less severe mucosal damage, as well as the healthy control group ($p=0.001$) Tables 1 and 2, respectively. Throughout the current study, the decrease in the concentrations of iron in sera of patients with CD agrees with the results reported previously [21- 24].

The current result is also compatible with other results [25, 26], which concluded that such low iron values may due to the diminished absorption of this metal by the proximal small intestine site, which is the greatest damage site in celiac disease. Andrews, 2004 reported that several factors affect iron absorption from this site; among them are the intestinal acidity and the intact mucosal surface [26]. Balaban *et. al.*, 2019 [27], hypothesized three main mechanisms for iron deficiency pathogenesis in CD patients which are:

1. Reduced food intake which cause a decreased oral iron intake. This phenomenon is affecting the children during the first years of their life and is common in CD patients with typical forms of the disease, where the prevalent symptoms are anorexia and vomiting.
2. Iron malabsorption is caused by diseases that affect the small intestine mucosa brush boundary or a decrease in the available absorptive surface.
3. An increase in small intestinal iron losses. The fast enterocyte turnover rate that results in changes to the epithelial-cell barrier or the intestinal micro erosions that happen as a result of the chronic inflammation might both be responsible for these phenomena.

Serum TIBC represents the maximum concentration of iron that can be bound by serum protein. Also, TIBC is highly correlated with serum transferrin [28]. Throughout this study, a significant increase in TIBC and UIBC concentrations was observed for severe histopathological mucosal damage celiac patients in Marsh III especially for those patients with total villous atrophy (Marsh III c), Table 1 and Table 2 respectively. This finding agree with a study done by Cook, 2005 who suggested that the iron deficiency anemia IDA patients are characterized by having a low serum iron and ferritin levels and elevated TIBC [29], while disagree with Souroujon *et al.*1982 results, which reported a non-significant differences in serum iron and TIBC of celiac patients, such variance in the results may be attributed to the differences in number, age and gender of the patients [30].

As compared to the equivalent control group, a non-significantly lower serum transferrin concentration was noticed in the patient group with partial, subtotal, and whole villous atrophy (Marsh III a, b, and c) and in the patient group with Marsh 0, I (Table 1) (a less severe histopathological mucosal change). This finding corroborated that serum transferrin could not be utilized as a meaningful measure to assess the iron storage of celiac participants in their 1987 study by Bonamico *et al.* [21]. A significant increase ($p = 0.027$) in serum transferrin level was obtained in patients with (total villous atrophy: Marsh III c) and (partial villous atrophy: Marsh III a) celiac patients' groups as compared with control group (Table 2). The observed significant increase in transferrin level among (Marsh III c) patients of total villous atrophy could be due to the progress stage of the disease interrelated with IDA, while the significant increase in transferrin in patient with partial villous atrophy (Marsh III a) may be due to the abnormalities in iron homeostasis because of the presence of inflammation beside the malabsorption [31, 32].

Contrary to what was found by Souroujon *et al.*, who found that there was no significant decrease in serum transferrin saturation percent TS (%) of those patients' who were on a normal diet, then transferred to a GFD, and again to a normal diet when compared to that of a healthy control group [30], the observed decrease in the transferrin saturation percentage was inconsistent with their findings.

Most patients with CD are anemic, that is due to the association between the iron overload genes (which cause hemochromatosis) and CD where the C282Y hemochromatosis-associated gene was reported to be more common in CD patients [33] .The other cause is that: there is a substantial variation in the extent of involvement of proximal small intestine in CD disease, that may allow for compensation [34].

Anemia is defined as a concentration of Hb< 12.0 g/dL in females and <13.0 g/dL in males [35]. And according to the World Health Organization (WHO), the individual with [Hb] corresponds to ≥ 9.5 g/dL is considered as having mild anemia, while who has a [Hb] ≥ 8 g/dL is considered to have a moderate anemia, but the one with [Hb] < 8 g/dL, is considered having a severe type of anemia. Iron deficiency anemia, usually manifests as microcytosis, hypo chromic anemia and patients characteristically have low serum iron, elevated TIBC and low ferritin. IDA in celiac primarily results from impaired absorption of iron, but there may also be occult blood loss in the gastrointestinal tract [36]. It is not surprising therefore that IDA is a common finding in the newly diagnosed patients with CD. The people who do not have anemia may have GFD. Several studies from North America and Europe suggested that IDA may be the sole manifestation of CD in the absence of diarrhea [37, 38]. The association of CD may be especially high in those who were unresponsive to oral iron therapy [39].

Moreover, anemia of chronic disease (ACD) was described in patients with CD [40]. It is well known that one of the essential factors in the pathogenesis of CD is the pro-inflammatory cytokines [41]. In this regard, both interferon-γ and interleukin-6 which are inflammation powerful mediators of hypoferremia, that lead to the iron homeostasis abnormalities are reported to be associated with ACD [42] Accordingly, Freeman [32] reported that in the majority of CD patients with anemia, the measured low serum ferritin was an indicator of IDA, where ferritin <30 µg/L in serum may be associated with IDA and if the range of ferritin concentration is 30–100 µg/L this may indicate the presence of (IDA + ACD). It is obvious from the results presented in Table 1, presence of a highly significant decrease (p=0.000) in serum ferritin levels among the CD group at (Marsh III a, b, c) than those at (Marsh 0, I) who were with a less severe mucosal damage and the control group.

In Iraqi patients with CD, the current analysis linked the degree of villous atrophy and ferritin levels. Individuals with subtotal/total villous atrophy had lower serum ferritin levels than those with partial villous atrophy; this result demonstrates the presence of an iron malabsorption situation and is consistent with Freeman's 2015 [32] findings.

Table 4 illustrates the correlation between the histological grading of the CD and the measured iron profile in the current study. The CD patients were divided according to the nature of their adopted food. Annibale *et al*., 2001 indicated that the impaired iron absorption in CD patients who were at a GFD, seemed to be the main_pathogenetical mechanism of anemia that resulted from iron deficiency that were measured in their studied CD patients and GFD alone could normalize the histological alterations that present in the intestinal mucosa of these [23]. Souroujon *et al.*1982 and Montoro-Huguet *et al*, 2021suggested the existence of a relationship between abnormal intestinal changes and low ferritin levels in CD with improvement in both when the patients were on a GFD [30, 43].

In general, it is understood that a male person consumes and eliminates roughly 1 mg of iron per day from a diet providing 10–20 mg, whereas women lose an average of 2 mg of iron per day in addition to highly variable menstrual losses that can vary from 4 to 100 mg each period. Women's demand for iron absorption is two times more than men's due to these iron losses. Harper *et al*. 2007 and Stefanelli *et al.* 2020, reported that only slightly more women (22%) than men (17%) presented with anemia in celiac patients (n=400, mean age=46.5 ± 16.2) [31 and 44]. This is in contrast to current analysis, in which: women with CD had a slightly frequency of iron deficiency at baseline as compared to men (19.3% vs. 8%) as shown in Figure 2. However, an Italian study reported that detection of anemia in their patients is presenting

complaint that lead to diagnose CD and this was much more frequently among women than men [22].

Conclusion

The majority of CD patients with iron deficiency anemia (29.7%) showed a considerable depletion of their iron reserves together with low hemoglobin levels. Iron deficiency was more common in Iraqi women with celiac disease than in males (19.3% vs. 8%). We emphasize the need of evaluating iron storage throughout CD and the initial stages of the GFD in order to determine whether and when to advise temporary iron supplementation.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental, health, higher education, and scientific research ministries in Iraq.

Conflict Ofinterest

The authors declare that they have no conflict of interest.

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