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Clinico-pathological study to evaluate the role of Hematoxylin and Eosin staining in determining the prevalence of celiac disease in patients with unexplained anemia who underwent endoscopic evaluation in Dhi Qar: a prospective study.

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ABSTRACT

Background: Celiac disease is an enteropathy of immune-mediated disease that is caused by a genetically determined persistent sensitivity to gluten. Conversely, iron deficiency anemia

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Keywords
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is the most prevalent type of anemia that people experience. A common extra-intestinal sign of celiac disease is IDA as well. **Objective**: This study aims to ascertain the sensitivity of endoscopy to diagnose celiac disease, as well as the prevalence of CD in patients with unexplained IDA and the GIT sources of anemia in those patients. Methods: This was a cross-sectional study of patients with IDA. Colonoscopy and Esophageo-Gastro-duedenoscopy were performed to determine the source of anemia. Four to six biopsies were collected from the second section of the duodenum. Small intestinal histologic characteristics were understood using the modified Marsh criteria. The total number of patients was 308, with 226 females and 82 men across all age categories. **Methods:** This was a cross-sectional study of patients with IDA. Colonoscopy and Esophageo-Gastro-duedenoscopy were performed to determine the source of anemia. Four to six biopsies were collected from the second section of the duodenum. Small intestinal histologic characteristics were understood using the modified Marsh criteria. The total number of patients was 308, with 226 females and 82 men across all age categories. **Results:** 78 of 308 individuals (25.3%) obtained biopsy results consistent with CD. 43 (55.1%) celiac disease patients were between the ages of 15 and 30; 18 (23%), between the ages of 31 and 45; 9 (11.5%), above the age of 45; and 8 (10.3%), under the age of 15. Out of 78 CD patients, 35 had Marsh grade IIIA, 23 had Marsh IIIB, 14 had Marsh IIIC, 5 had Marsh II, and one had Marsh I. Twenty were male, and fifty-eight were female. **Conclusion:** Even in women who are menstruation, CD should be taken into consideration by doctors as one of the potential reasons of anemia in any IDA patients of unclear origin. Premenopausal women with iron deficiency anemia should have serologic screening tests done, particularly if the anemia is not improving with oral iron therapy. In order to rule out CD, all patients with otherwise unexplained IDA should have upper gastrointestinal endoscopy and at least four duodenal biopsies.

Introduction

A chronic, small intestinal, immune-mediated enteropathy, celiac disease is brought on by dietary gluten in people who are predisposed to it. The combination of water-insoluble proteins found in rye,

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wheat, and barley that is detrimental to people with celiac disease is commonly referred to as gluten[1]. The small intestinal mucosa's villus atrophy, which is linked to poor nutrient absorption, clinical and histological relapse upon reintroducing gluten, and rapid clinical and subsequent histological improvement following rigorous adherence to a gluten-free diet (GFD), are indicators of celiac disease. The range and intensity of the mucosal lesion might vary greatly[2]. When severe untreated celiac disease is examined under magnification, the small intestinal mucosal surface reveals a flat mucosal surface completely devoid of normal intestinal villi. The quantity of epithelium surface that is accessible for absorption and digestion is reduced by these architectural changes[3]. A small intestinal biopsy is still the initial diagnostic technique used for people with celiac disease, which is a histological diagnosis in which there is unquestionably a clinical concern of the issue. There are some available tests for diagnosing celiac disease-related antibodies, and while they don't replace small intestine biopsies for identification, they may be useful as a diagnostic aid. In individuals who have a low index of suspicion for celiac disease or who are at high risk of improving the condition, positive antibody testing may help clinicians decide whether to perform a biopsy. A small intestinal biopsy is still the initial diagnostic technique used for people with celiac disease, which is a histological diagnosis in which there is unquestionably a clinical concern of the issue. There are some available tests for diagnosing celiac disease-related antibodies, and while they don't replace small intestine biopsies for identification, they may be useful as a diagnostic aid. In individuals who have a low index of suspicion for celiac disease or who are at high risk of improving the condition, positive antibody testing may help clinicians decide whether to perform a biopsy [7].

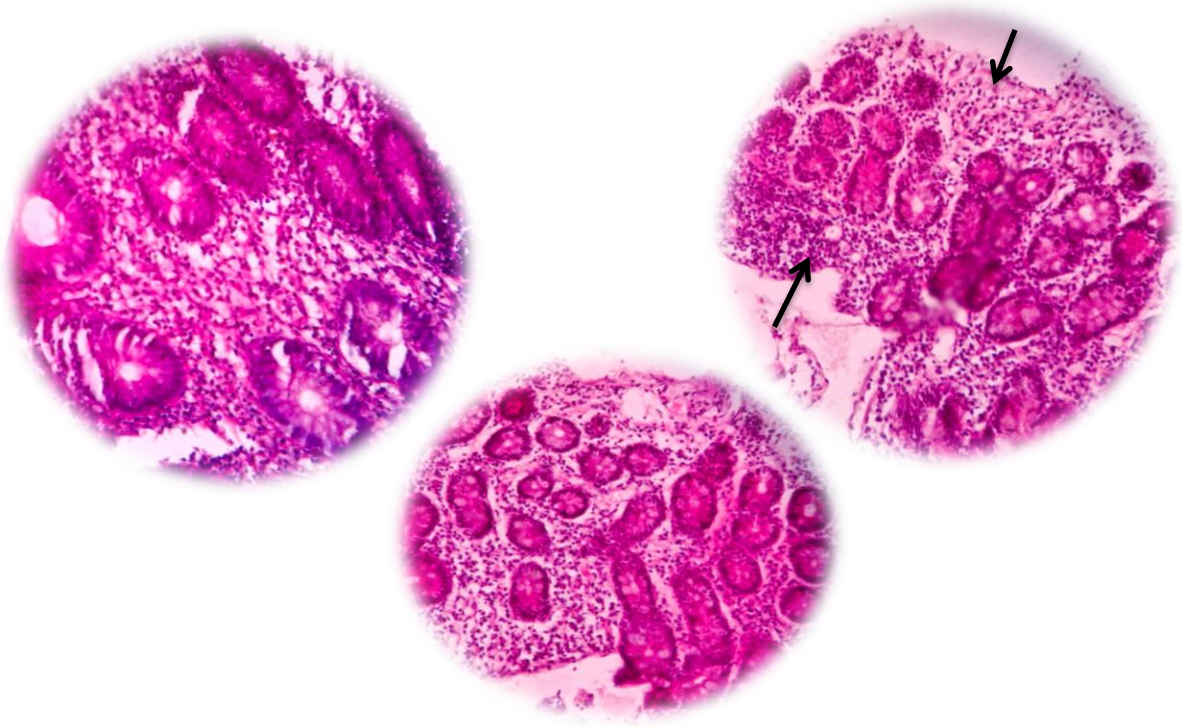

Figure 1. The modified March score is used to classify the severity of CD. It includes larger-than-normal depressions, more lymphocytes than usual, and villous atrophy, or the flattening and shortening of intestinal villi.

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Methods and Materials

Al-Nasiriya, the capital of the Thi-Qar governorate, was the site of a cross-sectional study. Between January and December 2018, every patient visited the gastrointestinal department of the Al-Hussein Teaching Hospital. For women, the inclusion criteria were Hb < 12 g/dl and/or serum ferritin <15 ng/ml, and for men, less than 14 g/dl and/or serum ferritin <25 ng/ml. Also, the blood film has hypochromic microcytic red blood cells (RBCs) and a mean corpuscular volume (MCV) of less than 80 fl.

Design of the study:

4-6 samples from the duodenum's second section were taken using endoscopic biopsy forceps for histo-pathological analysis. They were immediately fixed for four to six hours at room temperature in formalin solutions, and they were then routinely processed for standard histological analysis. The modified Marsh criteria were used to interpret small intestine histologic characteristics. The sample was sent to the central laboratory at the Al-Hussein Teaching Hospital to obtain the results of the duodenal biopsy, even though the hallmark histological features of proximal small bowel villous atrophy (either total or subtotal) with associated crypt hyperplasia and intraepithelial lymphocytosis are what are used to diagnose celiac disease.

Table 1. characteristic histological features of proximal small bowel villous atrophy

	Stage	Character [8,9]
1	Marsh 0	normal mucosal architecture, without significant intraepithelial lymphocytic infiltration
2	Marsh I	lymphocytic enteritis is normal mucosal architecture with a marked infiltration of villous epithelium by lymphocytes; marked is defined as more than 30 lymphocytes per 100 enterocytes
3	Marsh-II	lymphocytic enteritis with crypt hyperplasia) consists of intraepithelial lymphocytosis and elongation and branching of crypts in which there is an increased proliferation of epithelial cells
4	Marsh-III	comprises intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. There are three distinct stages of villous atrophy partial villous atrophy, the villi are blunt and shortened. Arbitrarily
	Marsh IIIA	, samples classified as partial villous atrophy if the villus-crypt ratio was less than 1:1
	Marsh IIIB	subtotal villous atrophy, villi are clearly atrophic, but still recognizable
	Marsh IIIC	total villous atrophy, villi are rudimentary or absent, and the mucosa resemble

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colonic mucosa

Once being gathered, all samples were placed in a formalin container, stained with Hematoxylin and Eosin, and examined under using a pathologist's light microscope. Histology showed lamina propria inflammation, villous atrophy, intraepithelial lymphocytosis (epithelial cells), and crypt hyperplasia, all of which were categorized as signs of celiac disease.

Pictures of Marsh stage findings of the studied sample

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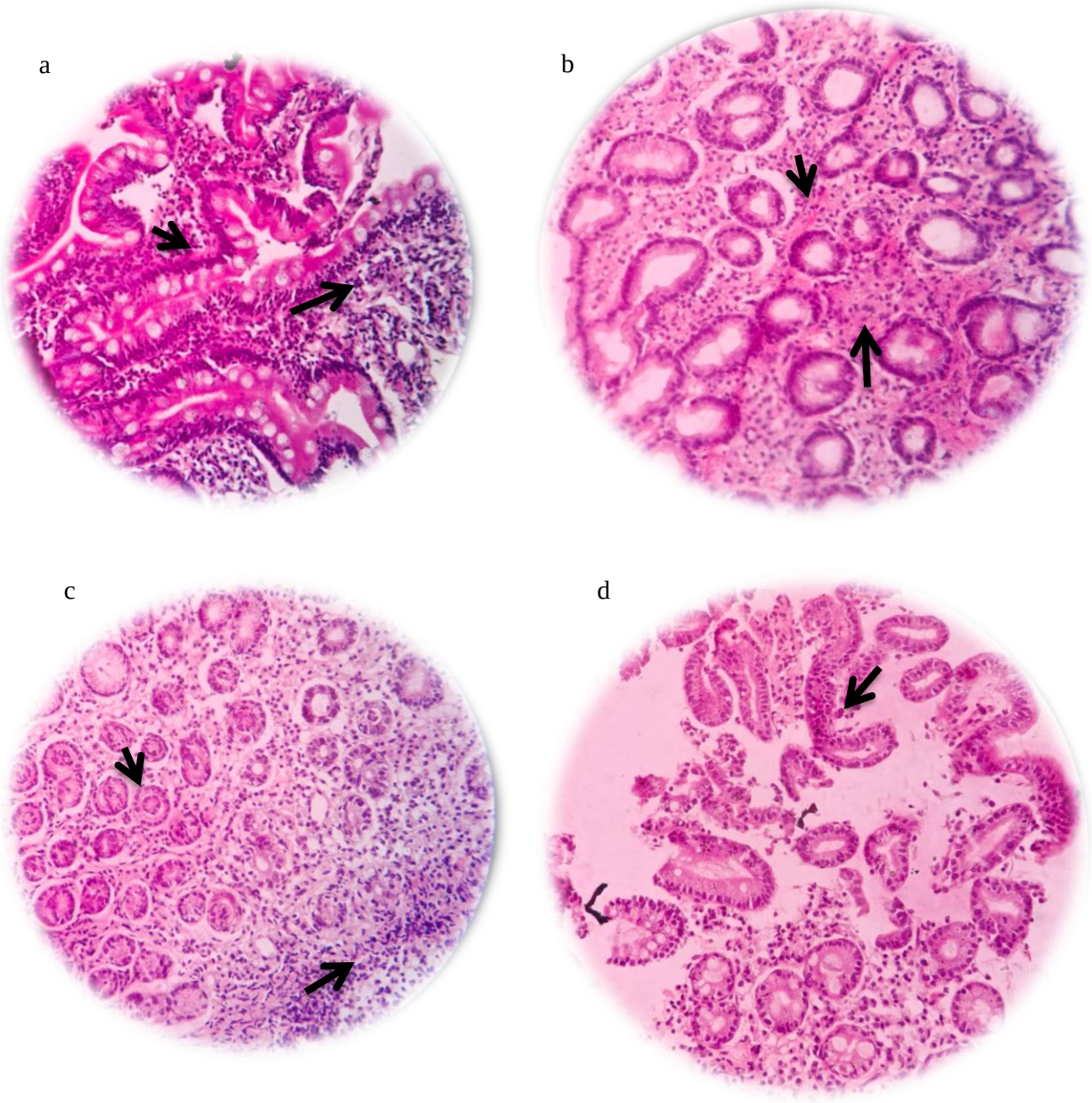

figure 2. (a) villus atrophy , (b) crypt Hyperplastic - (Figure showing proliferated colonic glands with surface epithelium showing serrated architecture (sawtooth appearance), no evidence of dysplasia (H&Ex100).(c)(d)(Serrated adenoma) Figure showing disordered proliferation of crypts with inverted T or L shaped crypt bases (boot shaped). The lining epithelium shows low grade dysplasia (H&Ex100)

Statistical analysis:

was carried out using the Statistical Package for the Social Sciences (SPSS) software version 25, the Pearson's chi-square test to ascertain the correlation between two categorical variables, the t-test to

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compare the means of quantitative variables, and the chi-square test to compare qualitative variables P < 0.05 was regarded as scientifically significant.

Results

About 340 of the 1763 patients who were referred from private clinics and hospitals and saw the gastrointestinal department at AL-Hussein Teaching Hospital in 2018 had unexplained IDA, according to this study.

Out of those 1763 patients, around 308 had unexplained IDA; 226 (73.4%) of these patients were female, and 82 (26.6%) were male, and they ranged in age. Only 86 of the age groups with unclear IDA brought celiac serology tests with them, and 72 of them had positive results (30 out of 72 had positive ATTG IgA, 13 had positive ATTG IgG, and 29 had positive for both tests), according to **table (1)**. Regardless of the serology results, OGD examinations were performed on all 308 individuals. **Table (2)** displays the endoscopic results of the OGD examination for 308 patients. **Figure 1** shows that 197 patients (64.0) had normal duodenum, while 111 patients (36.0%) exhibited serrated duodenal mucosa and scalloping folds, which are endoscopic characteristics of celiac disease. Other common findings on upper endoscopy included gastric erosions in 11 (3.6%), GERD in 7 (2.3%), gastric polyps in 5 (1.6%), esophageal web in 5 (2.3%), gastric ulcer in 4 (1.3%), diverticulae in 2 (0.6%), Brunner gland hyperplasia in 2 (0.6%), esophageal candidiasis, thick gastric mucosa, and gastric mucosal atrophy for each of the other findings. One patient had a stomach and duodenal ulcer (0.3%), and one patient each had esophageal varices, a GEJ lesion, and bulb duodenitis. Figure 2 illustrates one patient with a stomach and duodenal ulcer (0.3%), as well as one patient with esophageal varices, a GEJ lesion, and bulb duodenitis. In terms of 78 CD, 76 had serrated duodenal mucosa and only two had normal mucosa, indicating that endoscopy has a sensitivity of 97.4%. Celiac disease was detected during the biopsy in 78 individuals (20 males and 58 females), accounting for 25.3% of all patients with iron-deficiency anemia in this study. Of these, 55 had already tested positive for serology, and the rest were instructed to undergo testing after biopsy. The sensitivity of endoscopy is 97.4%. Of the 78 CD, 76 had serrated duodenal mucosa, and only two had normal mucosa. The biopsy revealed celiac disease in 78 patients (20 males and 58 females), accounting for 25.3% of all patients with iron-deficiency anemia in this study. Of these, 55 had previously tested positive for serology, and the remaining patients were recommended to undergo testing after biopsy, as shown in figures 3 and 4, respectively.

Table 1 : The frequency of age groups according to the gender in IDA Patients

Groups	Frequency	Male	Female	Percentage
Less than 15	14	6	8	4.5%
15 to 30	127	37	90	41.2%
31 to 45	95	17	78	30.8%
46 to 60	39	9	30	12.7%
Above 60	33	13	20	10.7%
Total	308	82	226	100.0%

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Table 2 : OGD findings in the IDA patients.

OGD findings	Frequency	%	Male	Female
Normal	115	37.3	22	93
Gastropathy	112	36.4	34	78
GERD	7	2.3	3	4
Haitus hernia	14	4.5	2	12
Duodenal ulcer	15	4.9	4	11
Gastric ulcer	4	1.3	0	4
Gastric erosion	11	3.6	5	6
Gastric &duod.ulcers	1	.3	0	1
Brunner gland hypertrophy	2	.6	1	1
Esophageal web	5	1.6	1	4
Gastric polyp	5	1.6	3	2
Diverticulum	2	.6	2	0
Bulb erosions	3	1.0	2	1
Gastric tumor	3	1.0	1	2
Esophageal varicose	1	.3	1	0
Thick gastric mucosa	2	.6	0	2
GEJ lesion	1	.3	0	1
Bulb duodenitis	1	.3	0	1
Esophageal candidiasis	2	.6	1	1
Gastric mucosal atrophy	2	.6	0	2
Total	308	100.0	82	226

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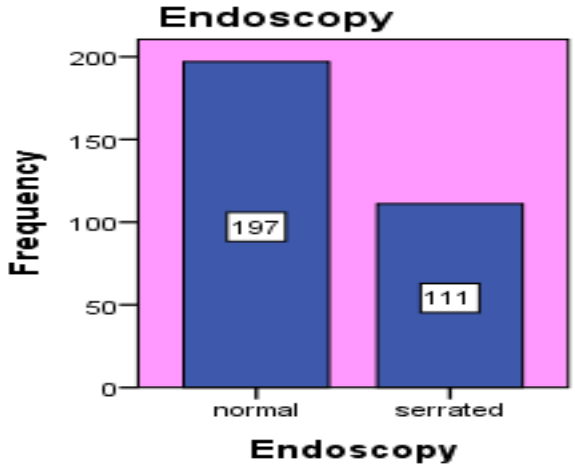

Figure 3 : endoscopic findings in the 2nd part of the duodenum in IDA patients

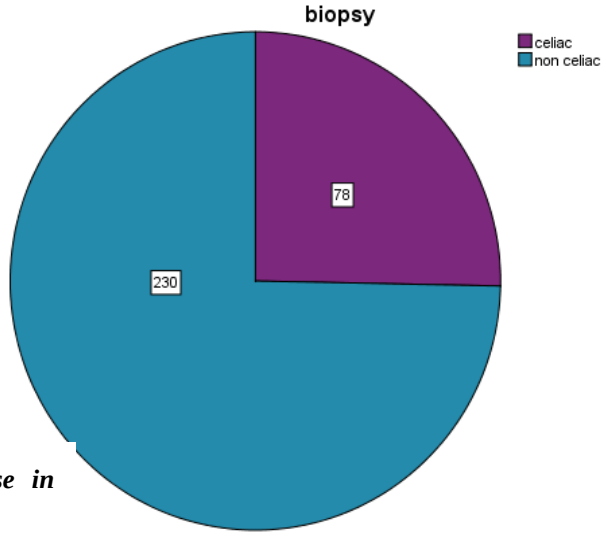

Figure 4 : ***the frequency of celiac disease in duodenal biopsy***

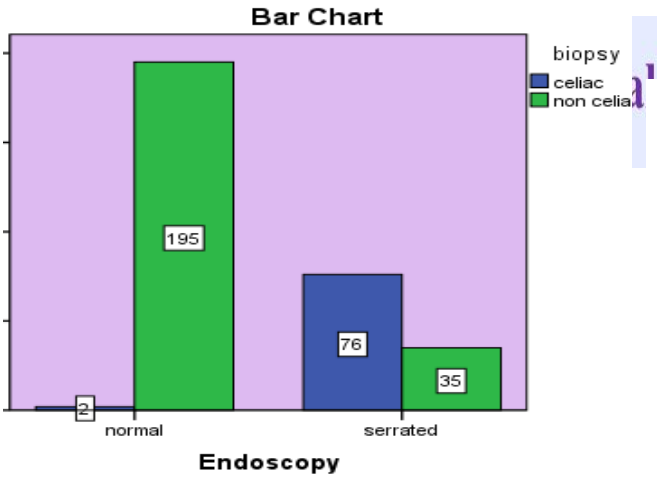

Figure 5:the frequency of celiac disease in comparison

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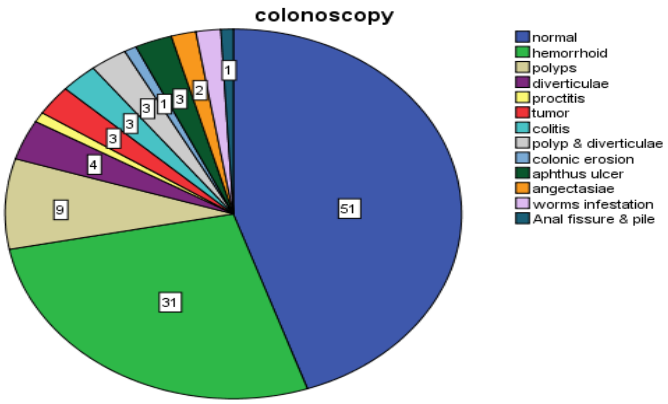

Figure 6 :the frequency of colonoscopic findings in IDA

The most frequent biopsy finding among those 78 patients was Marsh III A, which was found in 35 (44.9%) of them, followed by Marsh III B in 23 (29.5%), Marsh III C in 14 (17.9%) of them, Marsh II in 5 (6.5%), and Marsh I in 1 patient (1.2%). The prevalence of celiac disease in various age groups is displayed in table 3. As shown in Table 4, we also discovered that 12 patients exhibited positive endoscopic signs of celiac disease but negative biopsy results, which may indicate the possibility of celiac disease. We discovered that around six people had type 1 diabetes, one had thyroid illness, and one had Wilson disease and autoimmune hepatitis. The fact that the majority of patients did not exhibit skin rashes, diarrhea, or other symptoms of celiac disease highlights the disease's occult character.

Table 3 : the Marsh classification of celiac disease & frequency

Marsh classification	**Frequency**	**Percentage**
Marsh I	**1**	**1.2 %**
Marsh II	**5**	**6.5 %**
Marsh III A	**35**	**44.9 %**
Marsh III B	**23**	**29.5 %**
Marsh III C	**14**	**17.9 %**
Total	**78**	**100 %**

Table 4 : the frequency of celiac disease in different age groups

Age groups	**Frequency**	**Percentage**	**P value**
Less than 15	**8**	**10.3%**	**0.031**
15 to 30	**43**	**55.1%**	
31 to 45	**18**	**23%**	

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46 to 60	**8**	**10.3%**
Above 60	**1**	**1.2%**
Total	**78**	**100%**

Hemorrhoids were the most frequent colonoscopy results. According to table 5 Of the 114 individuals with iron-deficiency anemia, only 3 (2.6%) had colorectal cancer. There were 66 females and 44 males as seen on figure 5 and figure 6 respectively.

Table 5: sensitivity of Endoscopy according to biopsy

Endoscopy			Biopsy		Total	Pearson Chi-Square
			Celiac	Non celiac		
	Normal	Count	2_a	195_b	197	170.814
		Expected Count	49.9	147.1	197.0	0.001
	Serrated	Count	76_a	35_b	111	
		Expected Count	28.1	82.9	111.0	
Total		Count	78	230	308	
		Expected Count	78.0	230.0	308.0	

Each subscript letter denotes a subset of biopsy categories whose column proportions do not differ significantly from each other at the .05 level.

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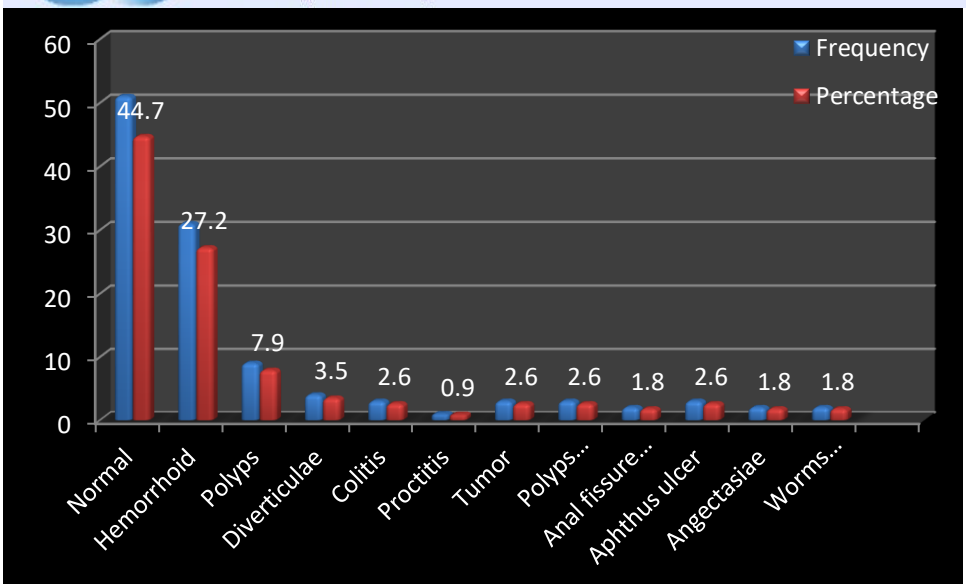

Figure 6 : Colonoscopy findings in 114 IDA patients.

Discussion

Our investigation revealed that, based on tTG (IgA and IgG) titer and biopsy data, the frequency of CD in IDA patients in Thi- Qar was 25.4%. Twenty-four percent of the 47 IDA patients in a research conducted in Al-Faiha Basrah had CD. According to research conducted in the USA and Turkey, the prevalence is 8.7% and 8.33%, respectively. In a study of 4120 IDA patients in Tehran, Iran, 14.6% of the patients had CD. This disparity in prevalence was caused by variations in CD's local prevalence and patient selection standards. In cases where gastrointestinal symptoms are vague or nonexistent, clinicians fail to rule out CD as the cause of IDA.[8] In line with earlier results that indicate the majority of CD cases in IDA are silent or atypical and are only identified by a screening process, a significant portion of IDA patients (54.8%) with CD in this study did not report any particular gastrointestinal symptoms. The majority of patients (74.4%) were female, which is consistent with findings from prior studies carried out in the United States and Iraq [9]. Out of the 78 CD patients in our study, 55 (70.5%) were premenopausal women, and the majority of them were in the 15–30 age range. Due to menstruation loss, premenopausal women have a higher need for iron. According to studies that used small-bowel biopsies and serologic testing on patients submitted for evaluation of IDA, CD was found in 1.8%–14.6% of individuals. This prevalence might be higher in people who don't respond to oral iron therapy [10]. CD was shown to be 20% prevalent in a subgroup examination of patients who did not respond to iron replacement. In individuals with villous atrophy, a positive CD-specific serology (TTG, DGP, and EMA) confirms the diagnosis of CD. Although a positive serological test supports the diagnosis, no single test is 100% specific for CD, and laboratory-to-laboratory variations in diagnostic precision are significant[11]. Since those individuals are at least five times more frequently IgA deficient than healthy control subjects, the goal of using both IgA and IgG antibodies in the diagnosis of CD is to detect endomyseal or tissue trans glutaminase antibodies and IgA in order to perform an appropriate screening for CD. Through the use of

duodenal histology analysis and a highly sensitive screening test (the tTG antibody test), we were able to prove that IDA can be the sole presenting symptom of CD. Twelve of the patients in this study tested positive for the disease, and their histology showed normal or nonspecific duodenitis. Duodenosis A common ailment that affects 3.8% of a population who tests negative for celiac disease and is linked to infection (especially Helicobacter pylori) and immune system changes is lymphocytosis. To the best of our knowledge, this is the first study to look into the prevalence of CD in patients with IDA of unknown etiology in the Thi-Qar population. According to this report, the prevalence of CD in Thi-Qar 2018 is 4.3%. whereas 25.4% of IDA patients had CD. For instance, in certain facilities, a seropositive patient's diagnosis can be made based just on the presence of a few inflammatory cells in a normally developing epithelium [(12)].

Conclusion

The prevalence of CD was 4.4% in patients attending the gastroenterology unit and extremely high in IDA patients (25.4%). Duodenal biopsy is necessary to confirm the diagnosis of IDA, which may be surprisingly common due to celiac disease. The results showed that the majority of individuals had no gastrointestinal problems. Because intestinal lymphoma is easily treated, CD screening helps IDA patients avoid intestinal lymphoma, repeated treatment failure, and the necessity for other pointless investigations.

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