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THE VALUE OF ENDOSCOPIC BIOPSIES FROM FIRST AND SECOND PARTS OF DUODENUM IN THE DIAGNOSIS OF CELIAC DISEASE IN CORRELATION WITH A SEROLOGICAL TEST

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Abstract

Celiac disease is a chronic immune-mediated enteropathy of the small intestine caused by environmental exposure to gluten in genetically susceptible individuals. This study was conducted to evaluate and correlate the serological with histopathological findings of duodenal biopsies for the diagnosis of celiac disease.

Sixty-eight patients, 40 (59%) females whose ages ranged from 13-75 year (mean age 36.4 years), and 28 (41%) males whose ages ranged from 13-65 year (mean age 37.8 years), with symptoms of chronic diarrhea, weight loss, bloating and unexplained iron deficiency anemia, were tested for anti-tissue transglutaminase IgA tTG, and correlated with histopathological findings of duodenal biopsies obtained from 1st and 2nd parts according to modified Marsh's classification. Histopathological findings from the 1st and 2nd duodenal parts were also compared with each other.

The results of the 68 patients who were enrolled in the study showed that: 24(35.3%) patients tested positive for anti-tissue transglutaminase (titer >18U/ml), 37(55.8%) patients had positive histopathological changes (stage I–III). Twenty-three (33.8%) patients who had both positive anti tTG and histopathological changes were classified as a celiac disease. The sensitivity of 1st and 2nd parts of duodenal biopsies in detecting celiac disease were 83.7% and 100% respectively.

In conclusion; the histopathological changes from the 1st and 2nd parts of duodenum in detecting celiac disease were equally representative especially in stage IIIa, b, and c.

Introduction

Celiac disease (CD) is a chronic immune-mediated enteropathy affects the small intestine caused by environmental exposure to gluten in genetically susceptible individuals, which demonstrates improvement with a withdrawal of gluten from the diet[1]. It occurs in about 1% of the general population worldwide, although it still underdiagnosed[2].

Celiac disease can present at any age, with classical symptoms such as diarrhea, flatulence, weight loss, stunted growth in children, and non-classical symptoms such as anemia (iron deficiency or other anemias), infertility, ataxia, muscle weakness, and aphthous stomatitis[3-7].

The diagnosis of celiac disease is made by clinical presentation, typical antibody responses and duodenal biopsies appearance[8].

As the first choice, total immunoglobulin A (IgA) and IgA tissue transglutaminase (tTG) are the standard serological tests, IgA endomysial antibodies (EMA) if IgA tTG is weakly positive, and take into account utilizing IgG EMA, IgG deamidated gliadin peptide (DGP) or IgG tTG if IgA is deficient[9].

The HLA- DQ2 allele is identified in 90-95% of patients with CD, and HLA- DQ8 is identified in the remaining patients[10].

The IgA and IgG antigliadin antibody testing are no longer recommended

because of their lower diagnostic accuracy with frequent false positive results[11].

People with positive serological test results should be referred for endoscopic duodenal biopsies, since firm diagnosis of CD can only be established after duodenal biopsies[12]. Histological examination demonstrates a cellular infiltration of lamina propria consisting of plasma cells and lymphocytes. The number of intraepithelial lymphocytes is markedly increased (>30 IEL/100 epithelial cells). Histopathological changes can vary from normal mucosa with increased IEL to completely flat mucosa[13].

This study was conducted to evaluate and correlate the serological with histo-pathological findings of duodenal biopsies in the diagnosis of celiac disease.

Patients and methods

This is a cross-sectional study, conducted in Basrah (Southern Iraq) at Al-Sadir Teaching Hospital during the period from November 2014 to May 2016.

A total of 68 patients were included in the study, 40 (59%) females whose ages ranged from 13-75 year (mean age 36.4 years), and 28 (41%) males whose ages ranged from 13-65 year (mean age 37.8 years). The ages were divided into seven groups starting from (10-19 year) and ending into (70-79 year). Patients with specific symptoms of CD such as diarrhea, weight loss, flatulence, bloating, and non-specific symptoms of CD such as unexplained iron deficiency anemia, short stature and mouth ulcers were referred from the outpatient department or private clinics for upper GIT endoscopic duodenal biopsies.

Iron deficiency anemia was diagnosed by complete blood count, film and serum ferritin.

All patients were tested for IgA anti tTG titer by enzyme-linked immunosorbent assay (ELISA) test (model Biotech). An antigen- antibody reaction had been detected by a microplate spectrophoto-meter (ELISA), and the titer of anti tTG had been reported as negative (titer <12 U/ml), borderline (titer 12-18 U/ml), and positive (titer >18 U/ml). The positive titers were subdivided into 3 groups (titer 18-50 U/ml), (titer 50-100 U/ml) and (titer >100 U/ml).

All patients underwent upper GIT endoscopy with fiberoptic endoscopy (GIF type OX260 model Olympus EVIS LUCERA) after an overnight fasting under local xylocaine spray with sedation by midazolam intravenously if indicated.

From each patient, two biopsies were obtained from the first part of the duodenum at 9 and 12 o'clock sites, and four biopsies from the second part of the duodenum from four quadrants. The specimens from 1st and 2nd duodenal parts were fixed into two different tubes containing 10% formaldehyde solution and labeled as D1 and D2 respectively.

All specimens were kept in the tubes for one day and then were processed and embedded on edge in paraffin wax. Sections of 5 micrometers thick were taken and stained by ordinary stains (Hematoxylin and Eosin stains) then examined under a light microscope by two histopathologists who were blind for clinical features and serological markers. The specimen from the 1st and 2nd duodenal parts were examined and graded by the two histopathologists together, according to modified Marsh's classification. (Appendix).

Histopathological changes from both 1st and 2nd part were recorded and compared with each other and correlated with the anti tTG level.

Patients were diagnosed as having CD if they were having both positive anti tTG IgA antibodies and histopathological changes of stage I and above.

Appropriate statistical methods were used to assess sensitivity, specificity, false positive, and false negative results with the assistance of statistical package for the social science (SPSS) version 22. P value less than 0.05 was considered to be significant.

Appendix *Modified Marsh's classification of histological finding in celiac disease Coberhuber

Marsh type	IEL/100 enterocyte of duodenum	Crypt hyperplasia	Villous atrophy
0	<30	None	None
I	>30	None	None
II	>30	Increased	None
IIIa	>30	Increased	Mild atrophy
IIIb	>30	Increased	Marked atrophy
IIIc	>30	Increased	Total atrophy

IEL (intraepithelial lymphocyte)

Results

Sixty-eight patients, 40 (59%) females, 28 (41%) males, were enrolled in this study, and subjected to serological and histopathological examinations, for diagnosis of celiac disease (Table I).

Table I: Gender distribution in studied patients

Gender	Celiac disease		Total
	Positive (%)	Negative (%)	
Male	9 (32%)	19 (41%)	28
Female	14 (35%)	26 (59%)	40
Total	23	45	68

This table shows that, more than 50% of patients were females. About one-third of the studied males and females were CD positive respectively.

Almost half of the patients who had CD were in the age group between 40-49 and 50-59 year, and represent 26% and 26% of the total cases respectively, to be followed by patients in the age group between (30-39) years and represent 21.7% of the total cases. None of the patients between (60-79) years had CD (Table II).

Table II: Age distribution in the studied patients

Age group	Celiac disease		Total	
	Positive (%)	Negative (%)		
10-19	3 13%	4 8.8%	7	10.3%
20-29	3 13%	12 26.6%	15	22.1%
30-39	5 21.7%	14 31.1%	19	27.9%
40-49	6 26%	9 20%	15	22.1%
50-59	6 26%	2 4.4%	8	11.8%
60-69	0	3 6.6%	3	4.4%
70-79	0	1 2.2%	1	1.5%
Total	23	45	68	100%

The majority of CD cases presented with unexplained iron deficiency anemia which represents 43.4% of the total cases, followed by those with chronic diarrhea with or without weight loss which represents 26% of the total (Table III).

Table III: The presenting features of studied patients

Clinical presentation	Celiac Disease		Total
	Positive (%)	Negative (%)	
Chronic diarrhea with or without weight loss	6 (26%)	11 (24.4%)	17
Bloating and flatulence	5 (21.7%)	20 (44.4%)	25
Iron deficiency anemia	10 (43.4%)	12 (26.6%)	22
Short stature	1 (4.3%)	1 (2.2%)	2
Mouth ulcer	1 (4.3%)	1 (2.2%)	2
Total	23	45	68

Almost two third of patients had negative tTG, only three patients had a borderline tTG results, most of the patients with positive tTG had titers between 18-100 u/ml, only two patients had a titer of more than 100 u/ml (Table IV).

Table IV: Anti tTG values in the studied patients

Anti tTG values in u/ml	No.	%
<12	41	60.3
12-18	3	4.4
18-50	13	19.1
50-100	9	13.2
>100	2	3
Total	68	100

A positive histopathological changes were similar between 1st and 2nd parts of the duodenum, especially in stage III a, b, and c, that represent 16.2%, 4.4%, and 1.5% respectively. At stage zero thirty-one patients had negative histopathological changes from the 2nd part of duodenum, as compared with 37 patients from the 1st part, this difference is due to 6 positive cases (3 at stage I and 3 at stage II) detected from the 2nd part of duodenum which were not detected from the 1st part of duodenum (Table Va).

Table (Va): Histopathological changes from 1st and 2nd parts of duodenum according to Marsh's classification

Stage	1st part of duodenum		2nd part of duodenum	
	No.	%	No.	%
Zero	37	54.4	31	45.5
I	14	20.6	17	25
II	2	2.9	5	7.4
IIIa	11	16.2	11	16.2
IIIb	3	4.4	3	4.4
IIIc	1	1.5	1	1.5
Total	68	100	68	100

The pattern of histopathological changes between 1st and 2nd parts of the duodenum is very close to each other, but the overall difference is statistical significance (p value<0.0001) (Table Vb). The sensitivity of 1st part of the duodenum is 83.7%, the sensitivity of 2nd part is 100%, while the specificity of both is 100%.

Table (Vb) the comparison between the histopathological changes of 1st and 2nd part of the duodenum

Histopathology		2nd part of duodenum			Total
		Zero	I, II	III (a,b,c)	
1st part of duodenum	Zero (%)	31 (83.8)	5 (13.5)	1 (2.7)	37
	I and II (%)	0	15 (93.8)	1 (6.3)	16
	III (a,b,c) (%)	0	2 (13.3)	13 (86.7)	15
Total		31	22	15	68

Fissur exact test =74.277, p value < 0.0001

The correlation between tTG and histopathological changes are seen in Table VI.

Table VI: Correlation between tTG and histopathological changes

Test	Result	Celiac disease		Total
		Positive (%)	Negative (%)	
Anti tTG	Positive	23 (95.8)	1 (4.1)	24
	Negative	0	44 (100)	44
	Total	23	45	68
histopathology	Positive	23 (62.1)	14 (37.8)	37
	Negative	0	31 (100)	31
	Total	23	45	68
	P value < 0.0001			

This table shows that, about one-third of the studied patients had celiac disease, the sensitivity of tTG IgA antibody in detecting CD is 100%, while its specificity was 97.7%. The sensitivity of histopathological examination from 1st and 2nd parts of duodenum in detecting CD is 100%, while its specificity is 68.8%.

Discussion

This study showed that the percentage of CD among studied patients was 23/68 (33.8%). This study was similar to the study of Bashar AS & Sarkis KS[14] who recorded CD in 26/72 (36.1%) among their patients.

The female to male ratio in CD is accepted to be 2:1, and some studies have suggested being more equal[15]. The female to male ratio in this study was nearly 1:1.

Regarding the age distribution of CD patients, some studies found that the CD frequency is twice in children than in adults[16], while other studies showed adult prevalence become more frequent, sometimes reaching similar or even higher than that of pediatric[17]. In this study half of CD patients ages were in their forties and fifties, 52% of all CD patients, followed by the age of thirties 21.7% of all CD patients. This is because the majority of referred cases in this study were adults and elderly patients.

Patients with CD traditionally presented with malabsorption, such as diarrhea, weight loss and failure to thrive (classical CD), but recently the non-classical CD, and asymptomatic CD have gained prominence[1].

In this study the majority of CD patients presented with unexplained IDA 10/23 cases, (43.4%) of the total cases, to be followed by those with chronic diarrhea with or without weight loss, 6/23 (26%) of the total cases.

Anti tTG was positive in 24/68 (35.3%) of patients, 23/24 (95.8%) of them had positive histopathological changes, and were classified as CD patients. One patient out of 24 with positive anti tTG who had no histopathological changes, could be explained by either the patient might have latent CD or so the histopathological changes have not yet established, or patchy involvement of small bowel lesion[18].

Histopathological changes were positive in 37/68(55.8%) patients, 23/37(60.5%) of them had anti tTG positive and were classified as CD positive, while the others 14 with positive histopathological changes tested negative to anti tTG.

The 14 patients who had positive histopathological changes and negative serology could be explained by that these patients might have other causes of mucosal lesions[19], or these patients might be IgA deficient which occurs in 0.2% of the general population and 2% of CD patients, so they lack antibody against tTG, in such patients IgG- tTG or IgG-DGP can be used[20].

Some of these patients might also have seronegative CD. The titers of tTG antibodies correlate well with the degree

of mucosal damage, so the sensitivity of these antibodies declines with a lesser degree of histopathological changes[21,22].

This study has shown that a positive histopathological changes were similar between 1st and 2nd parts of the duodenum, especially in stage IIIa, b, and c. This was similar to a study of Sarkis S & Fatah A[23]. These findings are helpful in patients where biopsies can be obtained from the 1st part only because of reasons that the scope cannot be negotiated to the 2nd part of the duodenum.

In CD villous atrophy may be patchy, so multiple biopsies from the 1st and 2nd duodenal parts are recommended. Taking at least four biopsy specimens from the 2nd part is associated with doubling the diagnostic rate as compared with patients undergoing a lower number of biopsies (less than four)[24]. On the other hand, if biopsies are not obtained from the 1st part, one would miss 9%-13% of patients who have clear evidence of CD (The incremental yield is 9%-13% if biopsies were obtained from the 1st part and the distal biopsies were negative)[25].

Genetic testing HLA DQ2-DQ8 are helpful in selected circumstances, if a patient has equivocal duodenal biopsy findings, or in refractory patients who have been diagnosed as CD but not responding to a gluten-free diet. In these patients, if HLA DQ2-DQ8 are negative, the diagnosis of CD is excluded[25].

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