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CLINICOPATHOLOGICAL STUDY OF AN IRAQI PATIENTS GROUP SUSPECTED TO HAVE COELIAC DIASEASE

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ABSTRACT

As the diagnosis of CD is more than expected among children and adults in Iraq, this study was carried out to describe the clinical features, histological and serological correlations in an Iraqi patients group consisted of adults and children suspected to have CD, and to correlate the serological results with the intensity of mucosal damage. 314 patients (142 male, 172 female, mean age, 15 years, range, 1–72) were recruited in the study. All were suspected on clinical basis to have coeliac disease. Endoscopy and duodenal biopsy in addition to serological assessment were done. The duodenal biopsies interpreted histologically according to modified Marsh criteria and the sera were tested for antigliadin antibody (AGA), endomysium antibody (EMA) and anti tissue transglutaminase antibody (tTG). It has been shown that histopathology still constitutes the golden standard test for ultimate diagnosis of CD according to Marsh criteria. Detection of the presence of EMA and tTG antibodies were diagnostic for the disease (PPV was 100%), while AGA is of less important since its sensitivity, was 77.6%. CD may be a prevalent life-long gastrointestinal diseases in Iraq. The study showed that the clinical features of coeliac disease have changed, symptoms are often minor and the disease can even be clinically silent. Histopathology was the golden standard test for diagnosis of the disease. Detecting the presence of serum antibodies was almost diagnostic for clinically suspected coeliac disease in children and adults especially EMA and tTG.

INTRODUCTION

Coeliac disease is a syndrome characterized by damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barely, wheat and rye in genetically susceptible subjects. The presence of gluten in these subjects leads to self-continuous mucosal damage, whereas elimination of gluten results in full mucosal recovery. [1-3]

The clinical manifestations of coeliac disease are changeable in nature and vary markedly with the age of the patient, the duration and extent of disease, and the presence of extra-intestinal pathological conditions. In addition, to the classical gastrointestinal form, a variety of other clinical manifestations of the disease has been described, including atypical and asymptomatic forms. [4] Therefore, diagnosis of coeliac disease is extremely challenging and relies on a sensitive and specific algorithm that allows the identification of different manifestations of the disease. Serological tests developed in the last two decades provide a non-invasive tool to screen both individuals at risk for the disease and the general population. However, the current gold standard for the diagnosis of coeliac disease remains histological confirmation of the intestinal damage in serologically positive individuals. The keystone treatment of coeliac disease patients is a lifelong elimination diet in which food products containing gluten are avoided. [5-6] The disease should be detected as early as possible, because untreated CD is associated with many severe complications such as intestinal lymphoma or cancer and osteoporosis. [5, 7]

For the case finding there are highly sensitive and specific autoantibody tests available; antigliadin antibodies, endomysial antibodies (EMA), and tissue transglutaminase autoantibodies (tTG) tests correlate well with the small bowel mucosal findings. [8-9] The population based screening studies worldwide have shown that the overall

prevalence of celiac disease ranged from 1:500 – 1:100. [7, 10] However, in clinical practice the disease often remains underdiagnosed. The major problem in diagnosing celiac disease is the multifaceted clinical picture of the condition. [9-10]

We know little about the situation of CD in Iraq because few studies have been conducted in Iraq yet [11-14]. These studies showed that diagnosis of CD is more than expected among children and adults in Iraq, meanwhile, large numbers of cases could be misdiagnosed due to limitation of serological antibodies usage in our laboratories except AGA. The present study was carried out to (1) describe the clinical features, histological and serological correlations in an Iraqi patients group consisted of adults and children suspected to have CD, (2) correlate the serological results with the intensity of mucosal damage in both ages.

PATIENTS, MATERIALS AND METHODS

This study was performed on 314 patients (142 male, 172 female, mean age, 15 years, range, 1–72) attending Teaching Gastrointestinal Hospital of Medical City in Baghdad. These patients were referred from different medical centers in Iraq, because they were suspected on clinical basis to have coeliac disease. The project was approved by the Ethics Committee of College of Medicine, University of Baghdad, and local Ethics Committee of the GIT hospital and written informed consent was obtained from each adult or child's parent individual participating in this study.

All patients were subjected to a personal interview using especially designed questionnaire format. The questionnaire gathered information included age, sex, complaint, duration of symptoms, type of diet, family history, oesophagogastroduodeno-scopy (OGD) findings, histopathological findings, CD serological tests findings, and the final diagnosis.

All patients underwent upper gastrointestinal endoscopy (OGD) with an Olympus endoscope (GIF-V 70, Olympus, Japan). During the procedure, 3-5 biopsy samples from distal duodenum were obtained for histological analysis. Formalin-fixed biopsy specimens stained with hematoxylin and eosin were studied with the use of light microscopy Biopsies were interpreted by two expert pathologists who were not informed about the clinical status of the patients and interpreted small intestinal histological features, according to the Marsh classification according to the modified Marsh criteria: [15-16] Marsh I consists of raised intraepithelial lymphocytes (IELs) with >40 lymphocytes per 100 enterocytes, Marsh II consists of raised intraepithelial lymphocytes and crypt hyperplasia, Marsh IIIa partial villous atrophy, Marsh IIIb subtotal villous atrophy, and Marsh IIIc total villous atrophy.

Venous blood samples were obtained from each patient, and sera subjected to anti gliadin antibodies (AGA), endomysial antibodies (EMA) and anti- tissue transglutaminase antibodies IgA (tTG) tests. Serum IgA EMA was detected qualitatively by indirect imunofluorescent (IIF) method using commercial slides of monkey esophagus (from Medic Company. Italy), with reticular staining of the muscularis mucosa at serum dilution of 1:3 reported as positive. However, AGA and tTG were performed by enzyme-linked immunosorbent assay (ELISA) in duplicate and according to the manufacturers' instructions. Negative sera, for all antibodies in highly suspected coeliac patients were subjected to the test with IgG monoclonal conjugate by IIF to exclude IgA deficiency disease associated with coeliac disease.

Diagnosis of coeliac disease was dependant on the presence of *Marsh III* only in histology examination. Any report, which did not include the features of Marsh III was considered as non-coeliac patient. Other diseases associated with chronic diarrhoea and abnormal mucosal morphology were excluded by careful clinical and laboratory assessment of each case, including careful examination of the stool to exclude parasitic or bacterial infection also by radiological and ultrasound investigations.

Statistics

Analysis comprised of summary statistics for gender and age. Data were analyzed using SPSS v10 for Windows and paired *t*-tests were used to compare the change in histopathology findings (Marsh grade). Data values were adjusted for age and initial values. Analyses where the *P*-value was <0.05 were considered to be statistically significant.

RESULTS

The results presented in this study were based on the analysis of data on a total of 314 patients in whom coeliac disease was suspected on clinical grounds. Since children with CD differ from adults in certain aspects, many of the associations presented will be grouped into two categories; first, children (<18 years) second, adults (≥18 years).

1. Clinical profile

Among the 314 patients in whom coeliac disease was suspected on clinical grounds, the diagnosis was documented in 226 patients only, the remaining 88 were labeled as non-coeliac patients.

The diagnosis of 26 (29.5%) of non-coeliac patients was duodentitis, while 18 (20.4%) of them were diagnosed as giardiasis, 3 (3.4%) had primary intestinal lymphoma and 2 patients (2.2%) had Crohn's disease. The remaining 39 (44.3%) of non-coeliac group had a normal duodenal histology.

As shown in table 1, more females were affected by coeliac disease among children. The female to male ratio among childhood coeliac patients was 1.28: 1. Meanwhile, there was a slight male preponderance among the adult coeliac patients. The female to male ratio was 0.82 : 1.

Table1: Frequency distribution of coeliac patients according to gender and age group.

		No.	%	Female/Male ratio	P-value
Children	Male	68	43.9	1.28:1	0.122
	Female	87	56.1		
	Total	155	100		
Adults	Male	39	54.9	0.82:1	0.122
	Female	32	45.1		
	Total	71	100		

The data in table 2, showed statistically significant higher proportion of coeliac children had offensive diarrhoea (85.2%) as a complaint compared to (55.3%) among non-coeliac group. The same higher frequency of diarrhoea was noticed among adult coeliacs (42.3%, compared to 61.0% among non-coeliac group).

There was a statistically significant difference in the frequency of other complaints like weight loss, abdominal distension and recurrent mouth ulcers while there was a small and statistically insignificant difference in the frequency of other complaints like short stature, anemia, skin lesions, musculo-skeletal, between coeliac and non-coeliac group for both children and adults.

Clinical presentation			Coeliac patients		Non coeliac patients	
			No.	%	No.	%
Children	General	1. Short stature	28	18.1	7	14.9
		2. Weight loss	130	83.9	12	25.5
		3. Failure to thrive	38	24.5	11	23.4
		4. Delayed puberty	9	5.8	6	12.8
	Gastro-intestinal	5. Chronic diarrhoea	132	85.2	26	55.3
		6. Abdominal distention, discomfort	80	51.6	11	23.4
		7. Recurrent mouth ulcers	69	44.5	8	17.0
		8. Nausea & vomiting	17	11.0	3	6.4
	Extra-intestinal	9. Anemia	58	37.4	21	44.7
		10. Skin lesion	1	0.6	0	0.00
		11. Musculo-skeletal	7	4.5	0	0.00
		12. Ataxia	1	0.6	0	0.00
Adults	General	1. Short stature	3	4.2	2	4.9
		2. Weight loss	48	67.6	17	41.5
		3. Delayed puberty	9	12.7	2	4.9
	Gastro-intestinal	4. Chronic diarrhoea	30	42.3	25	61.0
		5. Abdominal distention, discomfort	50	70.4	13	31.7
		6. Recurrent mouth ulcers	31	43.7	0	0.0
	Extra-intestinal	7. Anemia	33	46.5	19	46.3
		8. Skin lesions	4	5.6	0	0.0
		9. Musculo-skeletal	25	35.2	5	12.2
		10. Ataxia	1	1.4	0	0.0
		11. Infertility	3	4.2	4	9.8
		12. Recurrent abortion	1	1.4	1	2.4
		13. Psychological	2	2.8	2	4.9

2. Histopathological profile

Table 3 shows the histological findings seen on examining the duodenal biopsy of coeliac patients. Duodenal biopsies revealed histopathological changes of coeliac disease (Marsh III) in 226 cases from 314 patients, 155 children and 71 adults.

Twenty six of 155 (16.8%) of coeliacs children showed histopathological changes of Marsh IIIa (partial villous atrophy), compared with 15 (21.1%) adult coeliac patients, while, 62 (40.0%) children and 25 (35.2%) adult patients showed Marsh IIIb changes (subtotal villous atrophy), finally 67 (43.2%) children and 31 (43.7%) adult coeliac patients showed Marsh IIIc changes (total villous atrophy). Among non-coeliac patients, none of the children and adults had changes of Marsh III but 15 children (31.9%) and 14 adult (34.1%) showed Marsh I changes (infiltration of inflammatory cells), while 13 children (27.7%) and 9 adult (22.0%), showed Marsh II changes (Marsh I + crypt hyperplasia), table 3.

Table3: Frequency distribution of coeliac and non-coeliac patients according to histopathological findings and age group.

	Histopathology	Coeliac		Non-coeliac	
		No.	%	No.	%
Children	Marsh I	0	0	15	31.9
	Marsh II	0	0	13	27.7
	Marsh IIIa	26	16.8	0	0
	Marsh IIIb	62	40.0	0	0
	Marsh IIIc	67	43.2	0	0
	Normal	0	0	19	40.6
Total		155	100	47	100
Adults	Marsh I	0	0	14	34.1
	Marsh II	0	0	9	22.0
	Marsh IIIa	15	21.1	0	0
	Marsh IIIb	25	35.2	0	0
	Marsh IIIc	31	43.7	0	0
	Normal	0	0	18	43.9
Total		71	100	41	100

4. SERUM ANTIBODIES

Tables 4, 5 and 6 show serum antibodies positivity according to the disease status, age group and to the type and severity of histopathological findings

Table 4, shows high proportion of coeliac children showed positive serum antibodies. We found (89% AGA), (87.7% EMA), and (91.6% tTG) were positive, compared to (17% AGA) (2.1% EMA), and (2.1% tTG) for non-coeliac children and this difference is highly significant statistically. Meanwhile, adults CD showed (66.2% AGA) (100% EMA) and (100% tTG) compared with (12.2% AGA) (0% EMA) and (0% tTG). These associations between positivity rates and disease status, age group were statistically significant.

There was a statistically significant positive trend between serum positivity rate and the severity of histological changes in our coeliac patients. The AGA positivity rate increased from as low as 18.2% for patients with Marsh II changes to as high as 85.1% for those with Marsh III changes, while the rate of EMA positivity increased from (4.5%) for patients with Marsh II to (98%) for those with Marsh IIIc. The same applicable for tTG antibodies rate which increased from (4.5%) for patients with Marsh II to (100%) for those with Marsh IIIc changes (table 5).

As shown in table 6, the PPV of the serological tests in coeliac disease was high ranging from (92.4%) for AGA to (100%) for EMA and (99.6%) for tTG in patients with a clinical suspicion of coeliac disease. Given a positive test of any (3) antibodies one can be (92.4%) to (100%) confident that he is dealing with a real case of CD in clinically suspicious patients. On the other hand, according to NPV of the serological tests; given a negative test in a clinically suspicious situation excludes coeliac disease with confidence of (64.8%) in AGA, (85.6%) in EMA and (88.9%) in tTG.

The sensitivity was much lower (77.6% in AGA) than (95.8%) in tTG and (93.8%) in EMA. If these tests were to be used in screening for CD in general, one can expect to find true coeliac patients in (78% if AGA was used), (94% if EMA or 96% tTG was used) of positive tested individuals.

Table 4: Serum antibodies positivity rates according to disease status and age group.

	Group	Positive AGA No.	%	Positive EMA No.	%	Positive t TG No.	%	Total	P-value
Children	Coeliac disease	138	89	136	87.7	142	91.6	155	0.00001
	Non-coeliac disease	8	17	1	2.1	1	2.1	47	
Adults	Coeliac disease	47	66.2	71	100	71	100	71	0.00001
	Non-coeliac disease	5	12.2	0	0	0	0	41	

Table 5: Serum antibodies positivity rates according to type and severity of histopathological findings

Histology	Positive AGA		Positive EMA		Positive tTG		Total	P-value
	No.	%	No.	%	No.	%	No.	
Marsh I	7	24.1	0	0	0	0	29	0.00001
Marsh II	4	18.2	1	4.5	1	4.5	22	
Marsh IIIa	33	80.5	27	65.9	31	75.6	41	
Marsh IIIb	74	85.1	84	96.6	84	96.6	87	
Marsh IIIc	78	79.6	96	98	98	100	98	
Normal histology	2	5.4	0	0	0	0	37	
Total	198	63.05	208	66.24	214	68.15	314	

Table 6: Comparison between the performance characteristics of AGA test, EMA test, and tTG test in the diagnosis for children, adults, and total coeliac patients

	Variable	Sensitivity	Specificity	PPV	NPV
Children	AGA	89.03%	82.98%	94.52%	69.64%
	EMA	87.72%	100%	100%	71.21%
	tTG	91.61%	97.87%	99.3%	77.97%
Adults	Variable	Sensitivity	Specificity	PPV	NPV
	AGA	66.2%	87.81%	90.39%	60%
	EMA	100%	100%	100%	100%
	tTG	100%	100%	100%	100%
Total patients	Variable	Sensitivity	Specificity	PPV	NPV
	AGA	77.6%	85.3%	92.4%	64.8%
	EMA	93.8%	100%	100%	85.6%
	tTG	95.8%	98.9%	99.6%	88.9%

PPV = positive predictive value, NPV = negative predictive value

DISCUSSION

This study demonstrates for the first time, to our knowledge that among 314 patients in whom CD was suspected on clinical grounds, the diagnosis was documented in 226 patients only, meanwhile, the remaining 88 were labeled as non-coeliac patients.

The sex distribution of coeliac patients showed a slight female excess in the present study, but this was not significant statistically for both children and adults. This was compatible to the results reported in other countries [3, 7, 17]

CD can present with a wide spectrum of Gastrointestinal and extraintestinal manifestations and will often be overlooked unless it is actively considered in patients with unexplained clinical and laboratory features or associated diseases [6]. The reasons why the clinical expression of CD is so highly variable and why presentation can occur at any time in life, from infancy to very old age, are not fully understood. Symptoms of malabsorption are usually more marked during the first years of life and then gradually decrease. The group of patients with CD in the present work was clinically heterogeneous (table 2). Some subjects had no symptoms and presented only with short stature, others had mild complaints as malodorous flatus, while severe offensive diarrhoea was the main presenting symptom in others, especially in children. This was also consistent with other studies [18-20]. The explanation is that in spite of the absence of typical coeliac disease symptoms, most of the CD subjects showed some features that could raise the suspicion about small bowel disease. A common feature of subclinical coeliac disease is the positive clinical response to the gluten-free diet, which was seen in apparently healthy subjects with minimal changes. [21-22]

The present work relied on the histopathological examination as the golden standard test for differentiating between coeliac and non-coeliac patients. CD diagnosis was confirmed when there were infiltration of inflammatory cells, mainly IELs, crypt hyperplasia and villous atrophy (Marsh III). [23-27] However, in individuals who were on normal diet and had normal small bowel villous architecture, they could still have gluten sensitivity. They may be a cases of a latent coeliac disease and they might contracted small bowel villous atrophy and crypt hyperplasia later in the disease process, other patients had villous atrophy, with no crypt hyperplasia; these changes are not specific for coeliac disease, so they were not included in the group of coeliac patient. These facts were accepted worldwide. [23-27]

In untreated coeliac disease, ingested gluten triggers the production of IgA serum antibodies (AGA, EMA and tTG) in the serum and tissue. These antibodies seem to be highly specific for CD. However, the AGA test is still the only routine serological test using in Iraqi hospitals as an indicator for CD. In the present study AGA, EMA, and tTG were used as followed worldwide [28-30]

AGA appears specific to detect gluten sensitivity rather than coeliac disease, since positive AGA was also seen in other diseases and normal people. The test is of less value in confirming a diagnosis of coeliac disease, if used as a single test, but it is good for monitoring diet therapy in established coeliac cases. [31] However, the higher sensitivity and specificity of EMA and tTG in this study are an important step and objective method in detecting CD before endoscopy approach.

The histological sub classification of Marsh III (Marsh IIIa, Marsh IIIb, and Marsh IIIc) in this study, showed the correlation between the severity of mucosal damage and the appearance of autoantibodies. The question is how many CD patients will be missed in screening programs that rely too much on serology. The problem of negative serology in untreated coeliac disease patients (19 cases EMA and 13 tTG in this study) is underestimated, and data about the subgroup with minor tissue damage are lacking in the literatures. In the majority of studies, the sensitivity of serological antibodies is evaluated in patients with severe villous atrophy and an intestinal biopsy has been suggested only in those cases showing at least one abnormality on serology [20, 21). Most likely a subgroup of non-symptomatic coeliac patients negative for EMA or tTG will be under diagnosed, especially those with partial villous atrophy (Marsh IIIa). [29-30] At present, there is no discussion in the literatures about serology negative coeliac patients. It is important to avoid a self-fulfilling prophecy, taking biopsies only from EMA or tTG-positive individuals. Small bowel biopsy should be the first diagnostic procedure when there is a clinical suspicion of coeliac disease, in spite of positive or negative results of serology. For small intestinal biopsy to be replaced by the serological testing method as the diagnostic test of choice for coeliac disease, a sensitivity and specificity approaching 100%) would be required. In order to reach this perfect rate, combination of serology tests must be done to the patient such as EMA with tTG. [32-33].

CONCLUSIONS

CD may be is one of the most prevalent life-long gastrointestinal diseases in Iraq, since a fairly large number of coeliac patients were collected in a period of 20 months at one hospital in Baghdad; capital of Iraq. This study showed that the clinical features of coeliac disease have changed, symptoms are often minor and the disease can even be clinically silent. Histopathology was the golden standard test for diagnosis of the disease. Detecting the presence of serum antibodies was almost diagnostic for clinically suspected coeliac disease in children and adults especially EMA and tTG.

CONFLICT OF INTERESTS

The authors declare that there is no personal and funding conflict of interests associated with work

AUTHORS' CONTRIBUTION

We declare that work was done by all the authors named in this article and all the liabilities pertaining to claims relating to the content of this article will be borne by the authors. Muhamed T Osman coordinated the study design and participated in the all laboratory work, data collection, analysis and drafted the manuscript. Sanaa A Al-Nasiry participated in the histopathology & serology work and data analysis. Makki H Fayadh participated in doing the patient's endoscopy and clinical work. Balsam I Taha participated in the serological work. All the authors read the final manuscript.

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