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Research Article

Evaluation of Celiac Disease Activity in Karbala City based on Serological and Pathological Characteristics

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Abstract

Background: Gluten-enteropathy or celiac disease (CD) is a well-known autoimmune gastroenteropathy. The disease incidence is globally like an iceberg, with many cases believed to be undiscovered in the community. The discovery and monitoring of the activity of the disease is still a dilemma worldwide. Soluble Interleukin-2 receptor (sIL-2R) is a mediator involved in the inflammatory process, including CD. This study aimed to assess the level of sIL-2R in association with duodenal histopathological changes in CD, together with other serological parameters in comparison with healthy individuals.

Methods: A forty-five patients (34 females and 11 males, with a female: male ratio is about 3:1 and 45 healthy persons as a control group were included in this cross-sectional study. The ELISA technique was used to evaluate the serum level of sIL-2R, anti-tissue transglutaminase (tTG) IgA, and anti-deamidated gliadin peptide (DGP) IgG. The duodenal histological changes were evaluated dependent to the Marsh grading system (MARSH). Statistical analysis using the SPSS system has been applied.

Results: The patients with CD had high levels of serum anti-tTG, DGP, and sIL-2R with significant differences in comparison with the control group ($P< 0.05$). Marsh grades showed of highly significant difference in correlation with the immunological markers.

Conclusions: Celiac disease can be monitored by immunological and pathological methods. The sIL-2R antibody is a good tool to assess CD activity and patient compliance.

Keywords: Gluten, Enteropathy, Celiac disease, sIL-2R, Marsh grading system.

Introduction

Celiac disease (CD) is an autoimmune disorder affecting the gastrointestinal tract, with both intra- and extra-intestinal manifestations, which was first recognized in the 1940s and was initially linked to the consumption of wheat [1-4]. The autoimmunity of this disorder was clearly related to the strong association with the human leukocyte antigen (HLA) and the inflammation-mediated villous atrophy of the small intestine [5-6]. The evoking factor is the ingestion of gluten protein in several cereals like wheat, oat, barley, and rye [7-9]. The exact incidence of CD is not well known, but it was estimated to be 1.4% globally [10]. The incidence is rising, and the undiscovered cases may reach up to 50% [11-12].

CD can be like a chameleon in presentation, and its diagnosis is not always an easy task. Biopsy of the small intestine with subsequent histopathological examination is the cornerstone in the diagnosis. Additionally, serological studies for the detection of anti-deamidated (DGP) and anti-tissue transglutaminases (tTG) antibodies are also helpful for the diagnosis of CD [13-15]. There are both intestinal and extra-intestinal manifestations of the CD. Epigastric pain, nausea, abdominal bloating, and features of malabsorption are the main intestinal-related symptoms of the disease [16-17]. Iron deficiency anemia and megaloblastic anemia take place during CD due to reduced absorption of iron and vitamin B12, which are the well-known extra-intestinal dilemmas that are seen both in adults and children having CD [18-19]. There are multiple variations in both HLA and non-HLA genes in the pathogenesis of CD. Persons carrying the HLA-DQ2 & DQ8 haplotypes are the most susceptible to have the disease, although such genetic variability is present in 30-40% of the general population. Frequently, the detection of this HLA typing is ordered for the exact diagnosis of CD [3, 20-21].

Soluble IL-2 receptor (sIL-2R) is mainly released in case of T-cell activation and is elevated in the serum in many immunological disorders and can be used to assess or predict different immunological diseases that lead to the release of the free component of the receptor into the serum [22-23]. IL-2 receptor is expressed in variable levels on various immune cells, including dendritic cells, antigen-presenting cells, and different T-lymphocyte cells. There are three types of sIL-2R: monodimeric, low-dimeric affinity, and trimeric high-affinity variants, all of which are stimulated by IL-2 cytokine [23-25]. Additionally, elevated serum levels of sIL-2R were observed in several infectious and neoplastic conditions [26-27].

This study is planned to assess the serum level of sIL-2R in patients with celiac disease in association with the histopathological changes, together with other serological parameters.

Materials and Methods

Patients

Forty-five adult patients (34 females and 11 males) with celiac disease were enrolled in this study. Their age ranged from nineteen to 60 years. The study was conducted at the AI-Imam AL- Hussein Medical City Hospital in Karbala in Iraq, from May 2024 to February 2025. Different parameters related to the patients were included, like age, gender, family history, and whether patients have other autoimmune diseases. Hemoglobin and serum ferritin for both the patient and control groups were measured. Hemoglobin levels from 12.5-16.5g/dl in female and 13-17.5 g/dl in males was considered normal values. Serum ferritin (30-300 ng/ml in males and 13-150 ng/ml in females) was considered within normal limits. Forty-five healthy individuals participated as a control group.

Inclusion criteria: Adults (older than 17 years) diagnosed with CD based on serologically and/or histopathologically characteristic features were included in this study. Other included patients are those with a family history of CD, celiac persons diagnosed with genetic tests (HLA tests), and ill adults with symptoms highly suspicious of CD.

Exclusion criteria: Excluded individuals include children and patients who refused to submit to the endoscopic procedure, suspicious individuals, but with no cut-off serological tests, patients with well-known inflammatory bowel diseases like Crohn's or ulcerative colitis, and patients with non-celiac wheat allergy.

Sample collection and diagnosis

Serological markers using both IgG and IgM for anti-tTG and deamidated gliadin were analyzed using EuroImmune ELISA kits (Germany)(Normal serum level: tTG< 20 IU/dl, and DGP <25IU/dl, while ELISA kit for serum sIL-2R was purchased from the Bioassay Technology Laboratory (China)(normal limit < 160 ng /L). Serological levels of anti-tTG and deamidated gliadin, which are 100 times the normal level, did not progress to an endoscopy procedure.

Histopathological Assessment

The process of studying duodenal biopsies using Hematoxylin-Eosin (H&E) stain was recommended for all cases of CD suspicion. This study uses the histological grading system of CD invented by Michel Marshat (MARSH). The grade classified the stae of biopsy into grade I (increased intraepithelial lymphocytes (IEL) only), grade 2 (increases IEL + Crypt hyperplasia), and grade 3 (above + villous atrophy (G3a: mild villous atrophy, G3b: moderate villous atrophy, G3c= sever villous atrophy)[28].

Ethical considerations

The study was conducted using the serum of the patients and their duodenal biopsies after obtaining their agreement. Ethical approval was obtained from the College of Medicine at the University of Kerbala (Approval No.231/2024). All data were handled in compliance with confidentiality and ethical research standards.

Statistical analysis

Statistical analysis was performed using the SPSS program. A t-test was applied, and the p-value was considered significant when it was less than 0.05(<0.05).

Results

Regarding the age, there was no significant difference between the patients and the control group ($p>0.05$) as illustrated in Table 1. The mean value for hemoglobin for the patient and control groups was 10.68±1.71 and 12.52±1.65 g/dl, respectively, with significant differences between the two populations ($p<0.05$). The mean value of the ferritin for the patient and control groups was 16.51±15.82 ng/ml and 42.63±39.41 ng/ml, respectively, with a significant difference between the two groups ($P<0.05$) (Table 2). The serum level of anti-tTG IgA with positive records were seen more in the patient group than the control with a significant difference ($p<0.05$) (Figure 1). The data in Table 3 shows that most patients have high abnormal values of anti-DGP, with a significant difference from the healthy individuals. In concern with the serum level of IL2R, there was about 46.7% positivity in the CD patients while it was only 17.8% in the healthy

individuals, with a significant difference between the two groups ($p<0.05$) as shown in Table 4. Regarding the histological changes in the patients with CD, there was no significant difference between the genders ($p>0.05$) in association with gender differences (Table 5). Table 6 shows the association of the three MARSH histological grades with the different serological parameters. There was a significant difference between histological grading with both anti-tTG and IL-2R (p-values were 0.009 and 0.001, respectively). This association was not found in cases of anti-DGP IgG ($p=0.854$). The histological variants illustrated in Figure 2 showed the normal duodenal tissue (A) and the MARSH grade (B).

Table 1: The age distribution of both patients and control groups.

Age interval (years)	Patients No. (%)	Mean± SD	Control No. (%)	Mean ±SD	p-value
10-19	2 (4.4)	33.86±10.79	5 (11.1)	30.28±10.35	0.112
20-29	17 (37.8)		22 (48.9)		
30-39	11 (24.4)		10 (22.2)		
40-49	9 (20)		4 (8.9)		
≥50	6 (13.3)		4 (8.9)		
Range	17-56		17-55		
Total No. (%)	45 (100%)		45 (100%)		

SD; Standard Deviation

Table 2: The anemia parameters for the patients and control groups.

Clinical manifestations	Patients No. (%)	Mean±SD	Control No. (%)	Mean±SD	p-value
Anemia					
Low Hb	26 (57.8)	10.68 ±1.71	16 (35.6)	12.52 ±1.65	0.034
Normal Hb	19 (42.2)		29 (64.4)		
Total No.	45 (100)		45 (100)		
Serum ferritin level					
Low	35 (77.8)	16.51 ± 15.82	17 (37.8)	42.63±39.41	0.0001
Normal	10 (22.2)		28 (62.2)		
Total No.	45 (100)		45 (100)		

SD; Standard Deviation, Hb: hemoglobin

Table 3: Serum anti-DGP IgG level in both patients and control

Anti-DGP IgG levels	Patients No. (%)	Mean of test value ±SD	Control No. (%)	Mean of test value ±SD	p-value
< 25 IU/ml	3 (6.7)	180.77 ±158.41	41 (91.1)	8.49 ±6.64	0.001
>25 IU/ml	42 (93.3)		4 (8.9)		
Total No.	45 (100)		45 (100)		

SD; Standard Deviation

Table 4: level of s.IL2R in patients and control groups

sIL-2R levels	Patients No. (%)	Mean of test value ± SD	Control No. (%)	Mean of test value ± SD	p-value
<160ng/L	24 (53.3)	198.14±146.21	37 (82.2)	121.05±130.01	0.003
≥160ng/L	21 (46.7)		8 (17.8)		
Total No.	45 (100)		45 (100)		

SD; Standard Deviation

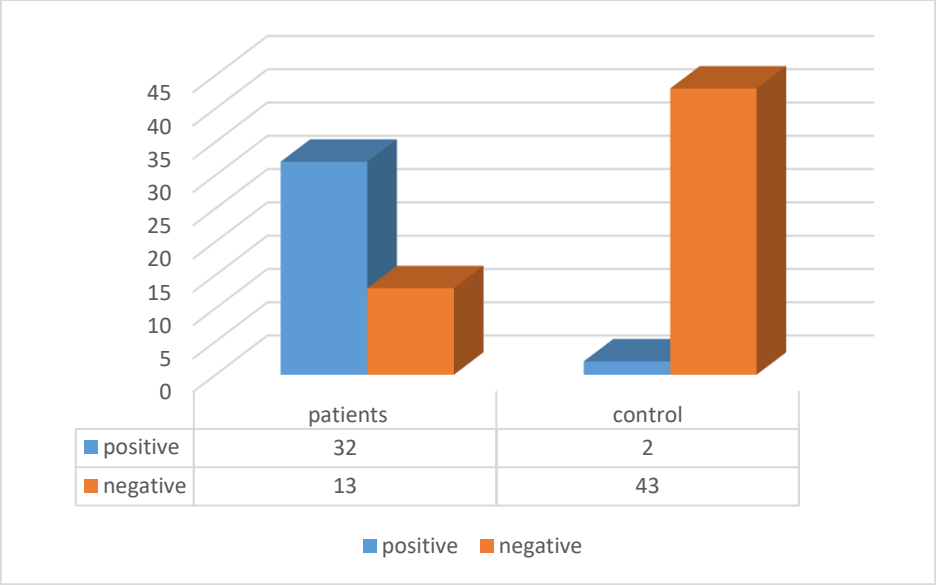

Figure 1: serum tTG IgA in patients and control groups

Table 5: Marsh grading with the gender differences

Gender	Marsh Grade I No. (%)	Marsh Grade II No. (%)	Marsh Grade III No. (%)	Total No. (%)	P-value
Male	7 (63.6)	2 (18.2)	2 (18.2)	11 (24.4)	>0.05
Female	15 (44.1)	9 (26.5)	10 (29.4)	34 (75.6)	
Total No.	22	11	12	45	

Table 6: Marsh grading with the serological markers

ELISA-detected parameters	Marsh G I No. (%)	Marsh G II No. (%)	Marsh GIII No. (%)	p- value
Anti-tTG IgA				
< 20 IU/ml	11 (50)	1 (9.1)	1 (8.3)	0.009
>20 IU/ml	11(50)	10 (90.9)	11 (91.7)	
Total No.	22 (100)	11 (100)	12 (100)	
Anti-DGP IgG				
< 25 IU/ml	1 (4.5)	1 (9.1)	1 (8.3)	0.854
>25 IU/ml	21 (95.5)	10 (90.9)	11 (91.7)	
Total No.	23 (100)	11 (100)	12 (100)	
sIL-2R				
<160 ng/L	5 (22.7)	9 (81.8)	10 (83.3)	0.001
≥160 ng/L	17 (77.3)	2 (18.2)	2 (16.7)	
Total No.	22 (100)	11 (100)	12 (100)	

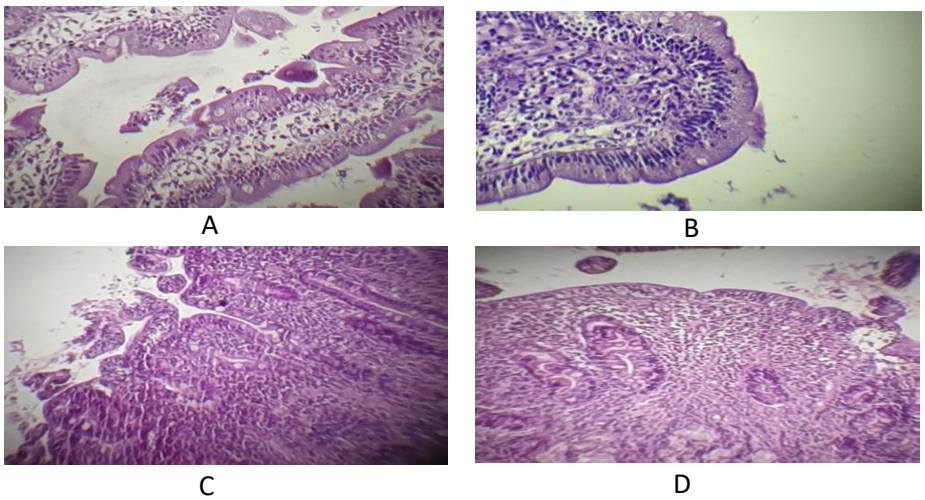

Figure 2: Histological variants based on MARSH grade
A= Normal, B=MARSH grade 2, C= MARSH Grade 3b, D= MARSH Grade 3c.

Discussion

Celiac disease is an autoimmune disorder that has variable intra-and extra-intestinal features. Anemia is a well-known complication of CD both in pediatric and adult groups [18]. Tissue transglutaminase is a calcium-dependent enzyme that plays a role in the immune-mediated pathogenesis of this gluten enteropathy [29]. The serum assay of anti-tTG in this study clarified that there is significant difference between patients and control groups, which is a fact that was proved by other studies [30].

Anti-deamidated gliadin antibody, another serological marker with high sensitivity and specificity for the diagnosis of CD, was used in this study, and the results demonstrated that it is statistically higher in the patients than in the healthy individuals, a result proved by Ortiz et al. (2019) [31]. The immune assay of sIL-2R can be used as a predictive method for CD, since it was clearly higher in the patients than in the control group in the present study. This result matches with results of other researchers [23, 32].

The histopathology of duodenal biopsies is considered as an important diagnostic tool in the discovery of CD [33]. The examination of biopsies from the patients enrolled in this study displayed different MARSH grading levels, and there was a significant difference among the different MARSH grading levels in relation to anti-tTG IgA and sIL-2R, but not with the anti-DGP IgG. Point may refer to the severity of tissue pathological changes may correlate with the rise of serological markers, as evidenced that proved by other authors [34].

Conclusions

Celiac disease diagnosis is not always an easy task, and applying other tools like the level of IL-2R serological test can be helpful. The severity of CD can be anticipated by calculating the serological level of sIL-2R. Serum level of sIL-2R can be a good tool for monitoring the level of a gluten-free diet in patients with CD. The severity of duodenal histopathological changes is well–correlated with the level of ELISA level of sIL-2R.

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Conflict of interest: The author declares that he has no conflict of interest

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