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Retrospective analyses of antibody titers in the diagnosis of pediatric Celiac Disease

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ABSTRACT

Objective: We aimed to evaluate the relationship between Tissue Transglutaminase IgA titer (tTGIgA) and Endomisium antibody (EMA) positivity and the stage of duodenal mucosal damage at Celiac disease (CD).

Material and Methods: The study group consisted of 233 children (2-18 years old) who were diagnosed with CD and admitted to our XXX Hospital, Pediatric Gastroenterology Outpatient Clinic, between September 2017 and November 2022. All patients underwent an endoscopy, and a histopathological diagnosis was made. In upper gastrointestinal endoscopy, one biopsy sample were taken from the duodenum bulb and four samples from the second part of the duodenum. Histological patterns were evaluated according to the Marsh-Oberhuber classification.

Results: A total 233 patients with CD were included in the study. The mean age of the patients at the time of diagnosis was 97.0 ± 57.1 months. The patients' mean tissue transglutaminase (tTG) IgA value was 172 ± 133. The most common Marsh-Oberhuber classification was found to be Marsh 3b (47.6%) in CD patients. According to the Marsh-Oberhuber classification, the mean tTGIgA values were significantly different compared to the groups.

Conclusion: We recommend starting a diet with a diagnosis of CD without endoscopy for patients with a tTGIgA value of 10 X ULN (upper limit of normal) or more recommended by ESPGAN, and we even support randomized prospective studies to reduce this value to 7-10 times or less.

Keywords: Celiac disease, Tissue transglutaminase IgA titer, Endomysial antibody

INTRODUCTION

Celiac disease (CD) is defined as 'an immune-mediated systemic disease caused by ingestion of gluten and related prolamins in genetically susceptible individuals (1). The main target of autoantibodies found in the serum of CD patients is tissue transglutaminase (tTG) enzyme. tTG deaminates the glutamine residues of gliadin peptides and converts them to glutamic acid. As a result of this modification, gliadin is negatively charged, binds with HLA-DQ2/DQ8 antigens and becomes recognized by T cells (2). Anti-tTG IgA titer was found to correlate well with biopsy staging (3). The IgA endomysial antibody test (EMA), which relies on fluorescence testing using primate esophagus or human umbilical cord, is based on antibody reactivity to tissue transglutaminase. Due to its high specificity, the EMA is well-suited as a secondary confirmation test (4).

Until the 1970s, CD was known as a rare disease affecting only children with a prevalence of less than 0.03% (5). The current worldwide prevalence of CD is approximately 1.4% based on serological testing and 0.7% based on biopsy findings (6). Because of the high prevalence of CD without typical symptoms, the use of serological diagnostic assays becomes extremely useful in clinical practice and eliminates unnecessary intestinal biopsies (7). The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) has recently revised its diagnostic criteria and recognized the critical role of serological testing in the diagnosis of CD (8).

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ESPGHAN also concluded that histology could be neglected in symptomatic patients with a high anti-tissue transglutaminase (tTG) IgA titer (10 times the upper reference limit) combined with the presence of EMA antibodies (8). Although current recommendations allow diagnosis without costly interventional procedures in many patients, it is discussed whether it is possible to increase the number of patients diagnosed without interventional procedures by using a lower threshold value. This study aimed to evaluate the relationship between anti-tTG titer and EMA positivity and the stage of duodenal mucosal damage at CD.

MATERIALS AND METHODS

A total of 233 celiac patients diagnosed in Adana City Training and Research Hospital between Semptember 2017 and November 2022 and in Kayseri City Training and Research Hospital between June 2021 and November 2022 were included. The age range of the patients was between 2-18. Patients diagnosed before 2020 were diagnosed according to the ESPGHAN 2012 guideline, and those diagnosed later were diagnosed according to the ESPGHAN 2020 guideline.

The patients were referred to the gastroenterology clinic due to gastrointestinal symptoms (diarrhea, weight loss, constipation, abdominal distension, nausea, vomiting, abdominal pain) and extraintestinal symptoms (iron deficiency) due to CD. Patients who were previously diagnosed with celiac disease, fed a low gluten diet for any reason, patients with selective IgA deficiency, patients with malignancy and Crohn's disease were excluded from the study. The study was approved by the local ethics committee of our hospital (Approval number : 2289, date: 01.12.2022). Written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Medical records of all patients were checked retrospectively. In upper gastrointestinal endoscopy, one biopsy sample was taken from the bulb of the duodenum and four samples from the second part of the duodenum. Samples were fixed in buffered formalin and embedded in paraffin wax. Standard sections were taken and stained with hematoxylin and eosin. Histological patterns were evaluated according to the Marsh-Oberhuber classification: type 0 indicates a normal histology, type I (infiltrative) is characterized by an increased number of IELs, type II (hyperplastic) also shows crypt hyperplasia, type III (destructive) also partial (IIIa), subtotal It is characterized by (IIIb) or total (IIIc) villous atrophy, and type IV is represented by villous atrophy alone (19). If there was clinical uncertainty about the diagnosis of CD, the patient was excluded from the study.

Total IgA, anti-tTG IgA and EMA values from serological tests were studied for all patients. Anti-tTG IgA titer was measured with Alisei model Seac device, EMA titer was measured with Aesku kit and Helmed device. Test results are expressed as IU/mL.

Quantitative values are expressed as mean and standard deviation, and qualitative values are expressed as percentages and proportions. Categorical data were compared using chi-square and Fisher's exact tests. Spearman correlation analysis test was used to evaluate the correlation between quantitative antibody and Marsh score results.

ROC analysis was used to achieve optimum sensitivity and specificity levels. Clinical status evaluation and gold standard diagnostic tests determined sensitivity, specificity, and positive and negative predictive values. Statistical analyzes were performed using SPSS version 21.0 (Statistical Package for Social Science; Chicago, IL). A value of $P < 0.05$ was considered significant.

RESULTS

233 patients with CD were included in the study. The mean age of the patients at the time of diagnosis was 97.0 ± 57.1 months. It was found that 64.8% of the patients were younger than 10 years old. 64.4% of the patients were female. The mean tTGIgA value of the patients was found to be 172 ± 133. EMA was found to be positive in 73.8% of the patients. The most common Marsh-Oberhuber classification was found to be 3b (47.6%) in CD patients. Demographic data and pathological characteristics of the patients are given in **Table1**.

Table 1. Demographic data and pathological characteristics of the study

	Patients (n= 233)
Age, Month, Mean ± StD	97.0 ± 57.1
Symptom duration, Month, Mean ± StD	16.4 ± 20.2
EMA, n (%)	
Negative	27 (11.6)
Positive	172 (73.8)
tTGIgA, Mean ± StD	172 ± 133
tTGIgA, n (%)	
Positive	205 (88.0)
Negative	28 (12.0)
tTGIgA, n (%)	
Negative	28 (12.0)
1-3 times	37 (15.9)
≥3-7 times	27 (11.6)
≥7-10 times	50 (21.5)
≥10 times	91 (39.1)
Gender, n (%)	
Male	83 (35.6)
Female	150 (64.4)
Range of age, n (%)	
0-119 month	151 (64.8)
≥120 month	82 (35.2)
Marsh-Oberhuber classification, n (%)	
1	5 (2.1)
2	2 (0.9)
3A	69 (29.6)
3B	111 (47.6)
3C	41 (17.6)
4	5 (2.1)

EMA: Endomysial antibody, tTGIgA: Tissue transglutaminase immunoglobulin A

According to the Marsh-Oberhuber classification, the mean tTGIgA values showed to differ significantly between the groups and are given in Table 2. According to Marsh-Oberhuber classification, EMA positivity, age group, gender, and EMA & tTGIgA status are given in **Table2**.

The Marsh-Oberhuber classification found it to be more common when both EMA & tTGIgA were positive in 3A, 3B and 3C. Mean tTGIgA values according to Marsh-Oberhuber classification are given in **figure1**.

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Table 2. Relationship between Marsh-Oberhuber classification and demographic data

	1	2	3A	3B	3C	4	P
tTGIgA, Mean ± StD	59 ± 29	100 ± 3	143 ± 131	180 ± 127	201 ± 151	284 ± 58	0,021
tTGIgA, n (%)							
Negative	0	0	14 (20.3)	10 (9.0)	4 (9.8)	0	0,001
1-3 times	3 (60.0)	0	10 (14.5)	17 (15.3)	7 (17.1)	0	
≥3-7 times	2 (40.0)	2 (100)	9 (13.0)	11 (9.9)	3 (7.3)	0	
≥7-10 times	0	0	18 (26.1)	27 (24.3)	5 (12.2)	0	
≥10 times	0	0	18 (26.1)	46 (41.4)	22 (53.7)	5 (100)	
EMA							
Negative	0	0	15 (25.9)	9 (9.7)	3 (7.9)	0	0,046
Positive	5 (100)	2 (100)	43 (74.1)	84 (90.3)	35 (92.1)	3 (100)	
EMA& tTGIgA							
N,N	0	0	11 (19.6)	3 (3.2)	2 (5.3)	0	0,045
P,N	0	1 (50.0)	3 (5.4)	7 (7.5)	2 (5.3)	1 (33.3)	
N,P	0	0	4 (7.1)	6 (6.5)	1 (2.6)	0	
P,P	5 (100)	1 (50.0)	38 (67.9)	77 (82.8)	33 (86.8)	2 (66.6)	
Age							
0-119 month	4 (80.0)	0	46 (66.7)	70 (63.1)	28 (68.3)	3 (60.0)	0,452
≥120 month	1 (20.0)	2 (100)	23 (33.3)	41 (36.9)	13 (31.7)	2 (40.0)	
Gender							
Male	4 (80.0)	0	21 (30.4)	48 (43.2)	9 (22.0)	1 (20.0)	0,024
Female	1 (20.0)	2 (100)	48 (69.6)	63 (56.8)	32 (78.0)	4 (80.0)	

EMA: Endomysial antibody, tTGIgA: Tissue transglutaminase immunoglobulin A, N: Negative, P: Positive

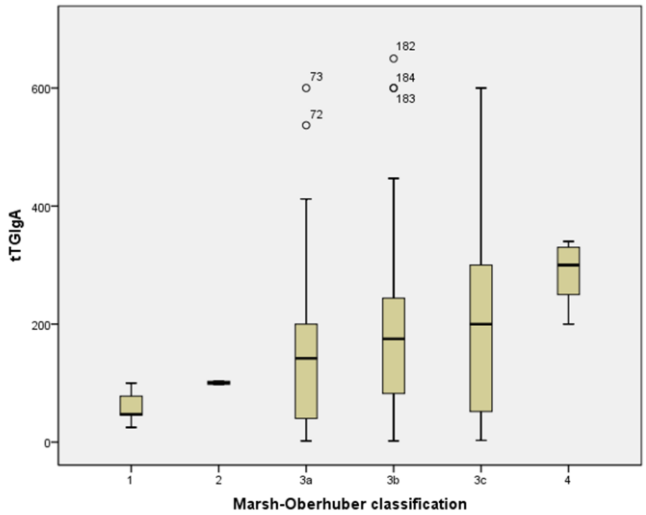

Figure 1. Mean tTGIgA values according to Marsh-Oberhuber classification

DISCUSSION

In this study, tTGIgA level was more than 10 times the upper limit in most celiac patients (39.1%) and Marsh-Oberhuber classification 3B at the time of diagnosis (47.6%) most frequently. The incidence of CD is increasing worldwide, possibly due to increased awareness of different clinical symptoms (12). Histopathology is the gold standard in the diagnosis of CD. However, in a multicenter study, 10% of the biopsies of the patients were found to be inadequate despite the interventional procedure (11). In addition, the weak correlation of histological examination among pathologists is another shortcoming. Due to these reasons, studies have been carried out recently on the diagnosis of CD in a less invasive way. The diagnostic value of serological tests has been studied. In this way, it is aimed to reduce the number of endoscopy, which is a more invasive procedure. The human recombinant tTG assay has been considered to have the highest diagnostic accuracy (9).

In the study of Alessoi et al., it was found that patients with anti-tTG positivity 7 times or more and found to be positive for EMA had 100% sensitivity and positive predictive value, that is, no patient without CD was found above this value (11). In another study with 671 CD patients, Anti-tTG IgA was positive in all patients, while IgA EMA was positive in 94.6% (13). Based on ROC curve analysis, anti-tTG serum levels were calculated as 3.6 times the cut-off value to identify CD patients with intestinal villous atrophy (AUC = 0.715, p<0.0001) (sensitivity = 76.8%, specificity = 63.2%, Youden index = 0.399, PPV = 97.2%, NPV = 14.0) (13). In a study of 144 serology-positive patients, 60% of the patients were diagnosed with CD disease, but normal endoscopic findings were found in 27% of the patients (14). For CD, the anti-TTG IgA titer was found to be 100% specific and 70% sensitive when the cut-off value of 150 was taken, but 84% specificity and 98% sensitivity when the Cut-off value was taken as 89.5 (14).

In a study evaluating serology tests, 34% false positive EMA, 17.5% false positive tTGA, EMA specificity 66%, and tTGA 83% specificity (16). When the role of the two serologies being positive in the study was investigated, it did not significantly improve diagnostic accuracy because PPV increased to 89% instead of 88% for tTGA alone (16). CD was diagnosed in 65.3% of patients with tTGA levels between 10 and 100 U/mL. In contrast, PPV of patients with tTGA level >100 U/mL was calculated as 97% (16). In the study conducted by Gülseren et al., the new cut-off value of anti-tTG IgA according to the ROC analysis was found to be 1.6 times (>32 IU/mL) of the commercial firm's threshold value of 20 IU/mL. Anti-tTG IgA susceptibility 100%; specificity was found to be 98.4% (5).

In 98% of CD patients, Marsh 3 lesions were detected on duodenal biopsy and CD was diagnosed (15). In the study of Meena DK et al., 70 pediatric patients with suspected CD were evaluated. The result was calculated to be statistically significant, showing that the mean anti-tTG titer increased with the severity of Marsh staging (2). In this study, when the cut-off value for anti-tTG was calculated with the ROC curve, an area under curve (AUC) of 0.068 in the curve, an anti-tTG value of 115 AU/mL at 6.4 times the upper limit of normal with p<0.0001 was 100%. It was found to have 76% sensitivity and 100% specificity with PPV and 17% NPV (2). Anti-tTG IgA levels and villous atrophy levels have decreased with age in CD patients (10). In the study conducted with CD patients with intestinal villous atrophy in 94.3% of the patients (98 patients type IIIa, 199 type IIIb, 333 type IIIc, and three type IV according to Marsh–Oberhuber classification), the intestinal villous structure of 5.7% of the patients was found to be normal. When the serum level of anti tTG is compared with duodenal lesions, it has been shown that as the grade of to Marsh–Oberhuber classification increases, the level of anti tTG increases (13). In the study, where the mean value of the t-TG level was shown to increase progressively with the increase in the histopathological lesion severity, there was a clear relationship between the serum level of t-TG and the histopathology of the small intestine, up to the point where the serum t-TG concentration was 3-5 times higher than the upper limit. has been shown (18). In our study, we found that when the Marsh-Oberhuber classification and tTGIgA values were compared, the values increased significantly (P= 0.021). As the Marsh-Oberhuber classification increased, the frequency of tTGIgA values 10 times and above the upper limit of the value increased gradually, and the result was found to be statistically significant (P= 0.001). We also calculated that approximately 60.6% of all celiac patients had a tTGIgA value of 7 times or more. As a result of our study, we recommend starting a diet with a diagnosis of celiac disease without endoscopy for patients with a tTGIgA value of 10 times or more recommended by ESPGAN, and we even support studies to reduce this value to 7 times or less.

In a study by Vermeersch P et al.; 4 different tTG IgA kits were tested. As a result, it was analyzed that a value >10 times above the threshold value was significant in detecting celiac disease in 4 kits, but there was great variability between kits for results between 3-10 times (9).

There are some shortcomings of our study. The first can be considered as the retrospective design of the study. The second important shortcoming is the absence of a control group. As it was a retrospective study, a control group could not be established. Despite these, the most crucial strength of our study is the sufficient number of pediatric patients in the homogeneous clinicopathological group.

CONCLUSION

It is recommended to start a diet with a diagnosis of celiac disease without endoscopy for patients with TGA-IgA values ≥10 times the upper limit of normal with appropriate tests and positive endomysial antibodies recommended by ESPGAN. However we support randomized prospective studies to reduce this value to 7 times or less.

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E**thical approval:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

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