



Association Between Tissue Transglutaminase IgA Levels and Villous Atrophy in Pediatric Celiac Disease: A Retrospective Cross-Sectional Study

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ABSTRACT

Objective: The 2020 European Society for Paediatric Gastroenterology, Hepatology and Nutrition no-biopsy algorithm for celiac disease (CD) requires a tissue transglutaminase IgA (tTG-IgA) level $\geq 10\times$ the upper limit of normal (ULN) plus endomysial antibody IgA (EMA-IgA) positivity on a second sample. This study evaluated the diagnostic performance of the tTG-IgA $\geq 10\times$ ULN threshold for predicting Marsh grade 3 histology at a center where EMA-IgA testing was not routinely available.

Methods: This retrospective study included pediatric patients who underwent duodenal biopsy for suspected CD between January and December 2023 at Şanlıurfa Training and Research Hospital. Patients were categorized as tTG-IgA $< 10\times$ ULN or $\geq 10\times$ ULN. Sensitivity, specificity, predictive values, accuracy, and Cohen's kappa were calculated using Marsh grade 3 as the reference standard. Factors associated with Marsh grade 3 were assessed using univariable logistic regression.

Results: Of 141 patients, 108 (76.6%) had tTG-IgA $\geq 10\times$ ULN and 130 (92.2%) had Marsh grade 3 histology. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for the threshold were 78.5%, 45.5%, 94.4%, 15.2%, and 75.9%, respectively. Agreement with Marsh grade 3 was low [$\kappa=0.125$; 95% confidence interval (CI), -0.040 to 0.290; $p=0.072$]. Any bulbar or distal duodenal endoscopic abnormality was associated with Marsh grade 3 (odds ratio =11.810; 95% CI, 2.697-51.703; $p=0.001$).

Conclusion: At our institution, without routine EMA-IgA testing, tTG-IgA levels $\geq 10\times$ ULN had high PPV for advanced histological injury; however, this marker alone was insufficient as a no-biopsy diagnostic criterion.

Keywords: Celiac disease, transglutaminases, biopsy, child, sensitivity and specificity, predictive value of tests

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INTRODUCTION

Celiac disease (CD) is a chronic, immune-mediated intestinal disorder caused by gluten intake in individuals with genetic susceptibility.¹ Diagnosis is based on the combined assessment of compatible clinical findings, celiac-specific serological antibodies, and enteropathy confirmed by duodenal biopsy.² Histopathological features, including intraepithelial lymphocytosis, crypt hyperplasia, and varying degrees of villous atrophy, are commonly graded using the Marsh classification. Because duodenal involvement may be patchy, one or two biopsy specimens from the duodenal bulb and at least four from the second portion of the duodenum are recommended to ensure accurate histological assessment.³

Although duodenal biopsy has traditionally been considered the reference standard for demonstrating celiac enteropathy, it is an invasive procedure. Its main limitations include the frequent need for sedation or general anesthesia in pediatric patients, the small but inherent risks associated with the procedure and anesthesia, its cost, and the additional burden on patients and their families.⁴ Furthermore, an insufficient number of specimens, sampling from inappropriate sites, suboptimal specimen orientation, and interobserver variability among pathologists may compromise the accuracy of histopathological assessment.⁵ These limitations have prompted the development of reliable no-biopsy diagnostic strategies for selected pediatric patients.^{2,4}



In the 2012 guidelines, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) established criteria allowing the diagnosis of CD without biopsy in selected children.⁶ The algorithm was simplified in the 2020 revision: a tissue transglutaminase IgA (tTG-IgA) level at least 10 times the upper limit of normal ($\geq 10 \times$ ULN), measured using an appropriate assay, together with endomysial antibody IgA (EMA-IgA) positivity in a second serum sample, was adopted as the principal serological criterion for a no-biopsy diagnosis. HLA-DQ2/DQ8 positivity and the presence of symptoms were no longer mandatory.⁷ In appropriate patient populations, this combined algorithm has been reported to have a positive predictive value (PPV) exceeding 99% for diagnosing CD.⁸

Nevertheless, implementation of the no-biopsy diagnostic algorithm depends on the availability of EMA-IgA testing in a second serum sample. EMA-IgA is assessed by indirect immunofluorescence, requires experienced personnel, and depends partly on operator interpretation. Consequently, limited availability and suboptimal interlaboratory standardization may hinder its routine use at some institutions, including ours.^{9,10} To address these limitations, studies across different age groups have investigated the diagnostic value of tTG-IgA level $\geq 10 \times$ ULN. In high-risk populations, this threshold has been reported to predict mucosal atrophy in up to 100% of cases.¹¹⁻¹⁴

This study aimed to evaluate the diagnostic performance of the tTG-IgA $\geq 10 \times$ ULN threshold in identifying Marsh grade

3 and thus villous atrophy in pediatric patients who underwent duodenal biopsy for suspected CD at a center where EMA-IgA testing was not routinely available. The secondary aims were to evaluate the agreement between the tTG-IgA levels $\geq 10 \times$ ULN and Marsh grade 3 and to identify factors associated with Marsh grade 3.

METHODS

Study Design and Study Population

This retrospective cross-sectional study included patients under 18 years of age who underwent upper gastrointestinal endoscopy for suspected CD at the Pediatric Gastroenterology Clinic of the Şanlıurfa Training and Research Hospital between January 1 and December 31, 2023. Patients were included if they underwent endoscopy for suspected CD and had duodenal biopsy specimens obtained in accordance with guideline recommendations.³ Patients were excluded if they had missing or unverifiable clinical, serological, or histopathological data; chronic diseases such as renal failure, cirrhosis, or malignancy; genetic disorders such as Down syndrome, Williams syndrome, or Turner syndrome; or selective IgA deficiency requiring the exclusive use of IgG-based serological tests. No patient had type 1 diabetes mellitus or autoimmune thyroiditis; these conditions were not included among the exclusion criteria. The patient selection process is shown in Figure 1.

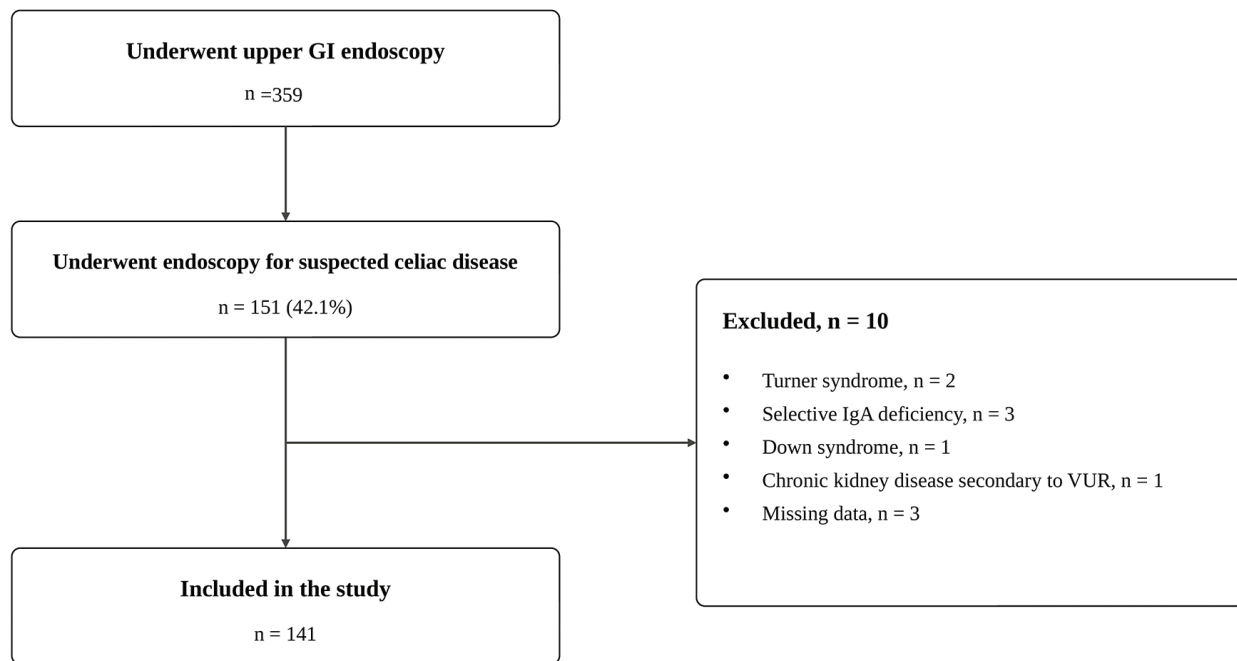


Figure 1. Flowchart of patient selection

The Harran University Non-Interventional Clinical Research Ethics Committee approved the study on April 14, 2026 (protocol no: HRÜ/26.01.01). The study was conducted in accordance with the Declaration of Helsinki, and all patient data were anonymized before entry into the study database.

Data Collection

Patients' demographic, clinical, anthropometric, serological, endoscopic, and histopathological data were collected from the hospital's electronic medical records and patient files. Age at endoscopy, sex, presenting symptoms and symptom type, age-standardized anthropometric z-scores, tTG-IgA levels, endoscopic and histopathological findings, and Marsh grade were recorded using a standardized data collection form.

Assessment of Clinical Findings

Presenting symptoms were obtained from patient records. Based on clinical presentation, patients were classified as having gastrointestinal symptoms, having extraintestinal symptoms, or being asymptomatic; the asymptomatic patients were identified through screening because they belonged to an at-risk group. Abdominal pain, diarrhea, constipation, abdominal distension, nausea and vomiting, and loss of appetite were classified as gastrointestinal symptoms. Given the heterogeneous and multisystem nature of extraintestinal manifestations in CD, all documented patient-reported manifestations other than the predefined gastrointestinal symptoms were recorded as such.

Anthropometric Assessment and Nutritional Status

Body weight and height measurements obtained within 1 month prior to endoscopy were used. Measurements were obtained according to standard procedures, using a mechanical scale and either a stadiometer or a length board, with the children wearing minimal clothing and no shoes. Anthropometric z-scores were calculated according to the World Health Organization (WHO) growth standards using WHO Anthro (version 3.2.2) and WHO AnthroPlus (version 1.0.4).

Wasting was defined as a weight-for-height (WFH) z-score below -2 standard deviation (SD) in children younger than 5 years, and as a body mass index (BMI)-for-age z-score below -2 SD in children aged 5 years or older.¹⁵ Stunting was defined as a height-for-age (HFA) z-score below -2 SD across all age groups.¹⁵

Serological Assessment

Serum tTG-IgA levels were measured in the hospital's central laboratory using an enzyme-linked immunosorbent assay with a commercially available ORGENTEC kit (Mainz, Germany), according to the manufacturer's instructions. The measurement range was 20-200 U/mL, and results outside this range were reported at the corresponding limits. According to the manufacturer, 20 U/mL was both the ULN and the threshold for seropositivity.

Patients with tTG-IgA levels $<10\times$ ULN (20 to <200 U/mL) were defined as Group 1, whereas those with levels $\geq 10\times$ ULN (≥ 200 U/mL) were defined as Group 2.

Endoscopic and Histopathological Assessment

All upper gastrointestinal endoscopies were performed by the same gastroenterologist in accordance with standard practice. Routine biopsy specimens were obtained from the esophagus, stomach, and duodenum, with additional targeted biopsies from any abnormal-appearing areas. For the histopathological assessment of CD, at least two specimens were obtained from the duodenal bulb and at least four specimens from the distal duodenum.³ Specimens from the two sites were placed in separate containers and sent to the pathology laboratory.

For each patient, specimens from the duodenal bulb were embedded in one paraffin block, and those from the distal duodenum were embedded in a second block. After trimming, two hematoxylin and eosin (H&E)-stained sections were obtained from each paraffin block. Histopathological examinations were performed by pathologists experienced in gastrointestinal pathology. If the mucosal architecture was not represented in a properly oriented vertical plane, the blocks were reoriented or the specimens were re-embedded, and additional sections were obtained. When necessary, immunohistochemical staining for CD3, CD20, and CD45 was performed to facilitate identification of intraepithelial lymphocytes.

For each biopsy site, a Marsh grade from 0 to 3 was assigned based on the specimen that showed the most pronounced histopathological changes. The higher grade identified from either site was used as the patient-level Marsh grade. When Marsh grade 3 changes were confined to the duodenal bulb, the condition was classified as ultra-short CD.¹⁶ In addition to H&E staining, Giemsa staining to detect *Helicobacter pylori* (*H. pylori*) was performed on concurrently obtained routine gastric biopsy specimens.¹⁷

Statistical Analysis

The normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed continuous variables are presented as mean $\pm$ SD, whereas non-normally distributed variables are presented as median and interquartile range [(IQR); 25th-75th percentiles]. Categorical variables are presented as counts and percentages. The groups with tTG-IgA levels $<10\times$ ULN and $\geq 10\times$ ULN were compared using Student's t-test or the Mann-Whitney U test for continuous variables, as appropriate and Pearson's chi-square test for categorical variables. Fisher's exact test was used when expected cell counts were low.

A 2 $\times$ 2 contingency table was constructed using tTG-IgA $\geq 10\times$ ULN as the index test and Marsh grade 3 as the reference standard. Sensitivity, specificity, PPV, negative predictive value (NPV), and

accuracy were calculated with 95% confidence intervals (CIs). Agreement between the tTG-IgA category and Marsh grade 3 was evaluated using Cohen's kappa coefficient.

Factors associated with Marsh grade 3 were assessed using univariable binary logistic regression. Because only 11 patients were in the negative outcome category, the recommended events-per-variable ratio ($\geq 10:1$) could not be achieved for a multivariable model. This limitation would have reduced the reliability of the estimated odds ratios (ORs). Therefore, multivariable analysis was not performed, and the univariable results are presented as descriptive, hypothesis-generating findings.

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). All tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Among the 141 patients, 97 (68.8%) were female and the median age was 7.9 years (IQR, 5.1-11.6 years). tTG-IgA levels were $\geq 10 \times$ ULN in 108 patients (76.6%). Overall, 131 patients (92.9%) were symptomatic. The most common clinical findings were abdominal distension and weight loss, each of which was reported in 82 patients (58.2%). Stunting was identified in 50 patients (35.5%) and wasting in 25 patients (17.7%).

At least one endoscopic abnormality in the duodenal bulb or distal duodenum was observed in 131 patients (92.9%). Bulbar findings included ulceration in 3 patients (2.1%), nodularity in 72 (51.1%), and atrophic mucosal appearance in 49 (34.8%). Distal duodenal findings included scalloping in 23 patients (16.3%) and mucosal fissuring in 106 patients (75.2%). Esophageal or gastric endoscopic abnormalities were observed in 60 patients (42.6%), including esophagitis in 2 patients (1.4%), gastritis in 44 patients (31.2%), and nodular pangastritis in 16 patients (11.3%). Duodenal biopsy findings were classified as Marsh grade 3 in 130 patients (92.2%). In 7 patients (5.0%), these changes were confined to the duodenal bulb and classified as an ultra-short CD pattern. *H. pylori* was detected in 31.9% of patients. The demographic, anthropometric, clinical, serological, endoscopic, and histopathological characteristics, as well as family history data, are presented in Table 1.

The proportion of female patients was significantly higher in Group 2 than in Group 1 ($p = 0.014$). No other clinical, anthropometric, endoscopic, or histopathological variables differed significantly between the groups (Table 2).

For predicting Marsh grade 3, the Group 2 threshold had a sensitivity of 78.5%, specificity of 45.5%, PPV of 94.4%, NPV of 15.2%, and overall accuracy of 75.9% (Table 3).

Among the 6 patients in Group 2 for whom Marsh grade 3 was not identified, 4 were classified as Marsh grade 2 and 2 were classified as Marsh grade 1. The patients with Marsh grade 2 were

Table 1. General demographic, clinical, and histopathological characteristics of the patients

	Value
Number of patients, n	141
Demographic characteristics	
Sex, female, n (%)	97 (68.8)
Age, years, median (IQR)	7.9 (5.1 to 11.6)
Age group, ≤ 6 years, n (%)	43 (30.5)
Age group, 7-11 years, n (%)	69 (48.9)
Age group, ≥ 12 years, n (%)	29 (20.6)
Family history	
Family history of celiac disease, n (%)	30 (21.3)
First-degree relative, n (%)	18 (60)
Second-degree relative, n (%)	12 (40)
Anthropometric characteristics	
HFA z-score, median (IQR)	-1.5 (-2.6 to -0.4)
BMI-for-age/WFH z-score, median (IQR)	-0.73 (-1.6 to -0.1)
Stunting, n (%)	50 (35.5)
Wasting, n (%)	25 (17.7)
Serologic characteristics	
tTG-IgA $< 10 \times$ ULN (Group 1), n (%)	33 (23.4)
tTG-IgA $\geq 10 \times$ ULN (Group 2), n (%)	108 (76.6)
Clinical symptoms	
Symptomatic, n (%)	131 (92.9)
Gastrointestinal symptom, n (%)	115 (81.6)
Abdominal distension, n (%)	82 (58.2)
Weight loss, n (%)	82 (58.2)
Abdominal pain, n (%)	72 (51.1)
Short stature, n (%)	49 (34.8)
Anorexia, n (%)	35 (24.8)
Diarrhea, n (%)	29 (20.6)
Constipation, n (%)	22 (15.6)
Nausea/vomiting, n (%)	20 (14.2)
Anemia, n (%)	10 (7.1)
Number of symptoms ≥ 3 , n (%)	84 (59.6)
Endoscopic findings	
Any bulbar or distal duodenal endoscopic abnormality, n (%)	131 (92.9)
Bulbar ulceration, n (%)	3 (2.1)
Bulbar nodularity, n (%)	72 (51.1)
Bulbar atrophic mucosal appearance, n (%)	49 (34.8)
Distal duodenal scalloping, n (%)	23 (16.3)
Distal duodenal mucosal fissuring, n (%)	106 (75.2)
Any esophageal or gastric endoscopic abnormality, n (%)	60 (42.6)

Table 1. Continued	
	Value
Appearance consistent with esophagitis, n (%)	2 (14)
Appearance consistent with gastritis, n (%)	44 (31.2)
Appearance consistent with nodular pangastritis, n (%)	16 (11.3)
Histopathological findings	
Bulb Marsh grade 1, n (%)	7 (5.0)
Bulb Marsh grade 2, n (%)	5 (3.5)
Bulb Marsh grade 3, n (%)	129 (91.5)
Duodenum Marsh grade 1, n (%)	14 (9.9)
Duodenum Marsh grade 2, n (%)	4 (2.8)
Duodenum Marsh grade 3, n (%)	123 (87.2)
Marsh grade 3, n (%)	130 (92.2)
Ultra-short celiac pattern, n (%)	7 (5.0)
Any additional non-celiac histopathological finding, n (%)	46 (32.6)
<i>Helicobacter pylori</i> positivity, n (%)	45 (31.9)
Data are presented as median (IQR; 25 th -75 th percentiles) or n (%), as appropriate. Patients may have more than one symptom or endoscopic finding. BMI: Body mass index, HFA: Height-for-age, IQR: Interquartile range, tTG-IgA: Tissue transglutaminase immunoglobulin A, ULN: Upper limit of normal, WFH: Weight-for-height	

symptomatic, positive for HLA-DQ2 and/or HLA-DQ8, and were diagnosed with CD after their antibody levels decreased and their symptoms improved on a gluten-free diet. The 2 patients with Marsh grade 1 were diagnosed with *H. pylori*-positive chronic active gastritis and received eradication therapy. During follow-up, antibody levels normalized in both patients.

Agreement between the tTG-IgA category (<10× ULN vs. ≥10× ULN) and Marsh grade 3 was 75.9% (107/141). However, Cohen's kappa coefficient was 0.125 (95% CI, -0.040 to 0.290; p=0.072), indicating slight agreement beyond chance (Table 4).

Potential factors associated with Marsh grade 3 were examined using univariable binary logistic regression. The presence of endoscopic abnormalities in the duodenum was associated with increased odds of Marsh grade 3 (OR =11.810; 95% CI, 2.697-51.703; p=0.001). Age, female sex, BMI-for-age/WFH z-score, HFA z-score, wasting, stunting, symptomatic status, tTG-IgA ≥10× ULN, the presence of other histopathological findings, a family history of CD and the number of symptoms were not significantly associated with Marsh grade 3 (all p>0.05) (Table 5).

DISCUSSION

In this study of 141 pediatric patients who underwent duodenal biopsy for suspected CD, we evaluated the diagnostic performance of the tTG-IgA ≥10× ULN threshold for predicting Marsh grade 3. The threshold yielded a PPV of 94.4%, with sensitivity, specificity, and NPV of 78.5%, 45.5%, and 15.2%, respectively. Despite the

high PPV of tTG-IgA ≥10× ULN threshold and advanced mucosal injury, concordance between the serological threshold and histopathological classification was low. These findings suggest that the tTG-IgA ≥10× ULN threshold may identify patients with a high probability of advanced histological injury. However, given the study design and the selected outcome measure, this threshold cannot, by itself, be considered sufficient to establish a no-biopsy diagnosis of CD.

The association between high-titer tTG-IgA and advanced mucosal injury has also been demonstrated in several retrospective studies of adults. Across different populations, tTG-IgA levels ≥10× ULN have been reported to predict villous atrophy in 95.2%-100% of cases.¹¹⁻¹³ The PPV of 94.4% in our cohort is broadly consistent with these findings. Nevertheless, Marsh grade 3 was not identified in 6 of the 108 patients in Group 2; however, 4 of these patients were later diagnosed with CD based on genetic, serological, and clinical assessments. This finding, along with the low kappa agreement, does not support using the ≥10× ULN threshold as a reliable standalone criterion for a no-biopsy diagnosis without confirmatory EMA-IgA testing. *H. pylori* infection may complicate the differential diagnosis between CD and non-celiac inflammatory duodenal injury. *H. pylori*-associated gastroduodenitis and peptic injury may cause duodenal intraepithelial lymphocytosis and, in some cases, crypt hyperplasia, resulting in histological changes that overlap with Marsh grades 1-2 in the absence of villous atrophy.¹⁸ Infection-related immune activation may also cause transient or false-positive tTG-IgA serology. In a pediatric cohort with positive celiac serology but with histology incompatible with CD, tTG-IgA levels decreased or normalized in 81% of infected patients after *H. pylori* eradication.¹⁹ Similarly, the 2 patients in our cohort with Marsh grade 1 and *H. pylori*-positive chronic active gastritis had normalized antibody levels after eradication therapy. These findings indicate that *H. pylori* infection should be considered when positive celiac serology is accompanied only by Marsh grade 1-2 changes, particularly in the absence of confirmatory EMA-IgA testing. In such cases, reassessment of celiac serology after successful eradication may prevent an incorrect lifelong diagnosis of CD.

The high PPV observed in our study should be interpreted in the context of the cohort's high pretest probability of disease. All patients had been referred for endoscopy and duodenal biopsy because of suspected CD, and the overall prevalence of Marsh grade 3 was 92.2%. Because PPV varies with disease prevalence, the high PPV observed in our study likely reflects both the discriminatory performance of the tTG-IgA threshold and the high probability of disease in this selected population. In a cohort from Buenos Aires, the tTG-IgA threshold required to achieve 100% PPV was 34 U/mL in the high-risk group but rose to 139 U/mL in the low-risk group, which included patients who presented with low-probability symptoms such as dyspepsia.²⁰ Therefore, the PPV observed in our study should not be generalized to populations with a lower pretest probability of CD.

Table 2. Comparison of clinical, anthropometric, and histopathological characteristics between Group 1 and Group 2

	Group 1 (n=33)	Group 2 (n=108)	p
Demographic characteristics			
Sex, female, n (%)	17 (51.5)	80 (74.1)	0.014
Age, years, median (IQR)	7.7 (5.9 to 11.4)	7.9 (4.8 to 11.6)	0.884
Family history of celiac disease, n (%)	8 (24.2)	22 (20.4)	0.634
Anthropometric characteristics			
HFA z-score, median (IQR)	-1.34 (-2.3 to -0.2)	-1.5 (-2.2 to -0.5)	0.577
BMI-for-age/WFH z-score, median (IQR)	-1 (-1.7 to -0.4)	-0.6 (to -0.1)	0.282
Stunting, n (%)	12 (36.4)	38 (35.2)	0.901
Wasting, n (%)	7 (21.2)	18 (16.7)	0.550
Clinical symptoms			
Abdominal distension, n (%)	19 (57.6)	63 (58.3)	0.931
Weight loss, n (%)	17 (51.5)	65 (60.2)	0.382
Abdominal pain, n (%)	13 (39.4)	59 (54.6)	0.131
Short stature, n (%)	10 (30.3)	39 (36.1)	0.542
Anorexia, n (%)	7 (21.2)	28 (25.9)	0.581
Diarrhea, n (%)	7 (21.2)	22 (20.4)	0.903
Constipation, n (%)	5 (15.2)	17 (15.7)	0.943
Nausea/vomiting, n (%)	5 (15.2)	15 (13.9)	0.782
Anemia, n (%)	0	10 (9.3)	0.121
Number of symptoms ≥ 3 , n (%)	18 (54.5)	66 (61.1)	0.501
Asymptomatic, n (%)	1 (3.0)	9 (8.3)	0.453
Gastrointestinal symptom, n (%)	28 (84.8)	87 (80.6)	0.578
Endoscopic and histopathological findings			
Any bulbar or distal duodenal endoscopic abnormality, n (%)	28 (84.8)	103 (95.4)	0.054
Any esophageal or gastric endoscopic abnormality, n (%)	13 (39.4)	47 (43.5)	0.675
Marsh grade 3, n (%)	28 (84.8)	102 (94.4)	0.129
Ultra-short celiac pattern, n (%)	3 (9.1)	4 (3.7)	0.354
<i>Helicobacter pylori</i> positivity, n (%)	10 (30.3)	35 (32.4)	0.820

Group 1: tTG-IgA $<10 \times$ ULN; Group 2: $\geq 10 \times$ ULN. Continuous variables are presented as median (IQR; 25th-75th percentiles) and were compared using the Mann-Whitney U test. Categorical variables are presented as n (%) and were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. BMI: Body mass index, HFA: Height-for-age, IQR: Interquartile range, tTG-IgA: Tissue transglutaminase immunoglobulin A, ULN: Upper limit of normal, WFH: Weight-for-height

EMA-IgA, detected by indirect immunofluorescence, is more specific for CD than tTG-IgA and provides complementary analytical and clinical confirmation in the diagnostic algorithm.²¹ During the study period, EMA-IgA testing was not routinely available at our institution, whereas pediatric endoscopy and histopathological assessment were established services. This local infrastructure should not be interpreted as evidence that endoscopy is generally more accessible than EMA-IgA testing or as a limitation of international diagnostic guidelines. Similar variability in the implementation of the ESPGHAN no-biopsy approach has been reported among pediatric gastroenterology centers in Türkiye.²² Accordingly, our findings are primarily

applicable to institutions with comparable local conditions. When EMA-IgA testing is unavailable locally, sending a second serum sample to a qualified reference laboratory is a reasonable alternative.

When EMA-IgA testing is unavailable, some clinical practice guidelines recommend repeating the tTG-IgA measurement in a second serum sample.²³ However, repeating the same assay may not increase specificity to the same extent as EMA-IgA, which employs a different analytical method. Moreover, patients may reduce their gluten intake after the initial test, potentially affecting the second serological result. Therefore, whether repeat tTG-IgA testing can substitute for EMA-IgA requires validation

Table 3. Diagnostic performance of the tTG-IgA $\geq 10\times$ ULN threshold for predicting Marsh grade 3			
	Marsh Grade 3 (+)	Marsh Grade 3 (-)	Total
tTG-IgA $<10\times$ ULN (Group 1)	28	5	33
tTG-IgA $\geq 10\times$ ULN (Group 2)	102	6	108
Total	130	11	141
Diagnostic criterion	Value	95% CI	
Sensitivity	78.5%	70.6-84.7	
Specificity	45.5%	21.3-72.0	
PPV	94.4%	88.4-97.4	
NPV	15.2%	6.7-30.9	
Accuracy	75.9%	68.2-82.2	
Cell values in the contingency table are numbers of patients. Diagnostic performance measures are presented as percentages with 95% confidence intervals. CI: Confidence interval, NPV: Negative predictive value, PPV: Positive predictive value, tTG-IgA: Tissue transglutaminase immunoglobulin A, ULN: Upper limit of normal			

Table 4. Agreement analysis between tTG-IgA category and Marsh grade 3	
Measure	Value
Raw (observed) agreement	75.9% (107/141)
Cohen's kappa coefficient (κ)	0.125
95% confidence interval	-0.040-0.290
p-value	0.072
Level of agreement	Very low/slight agreement
CI: Confidence interval, κ : Cohen's kappa coefficient, tTG-IgA: Tissue transglutaminase immunoglobulin A, The p-value was calculated for Cohen's kappa	

in prospective studies that include assays from different manufacturers, evaluate interlaboratory variability, and enroll different clinical risk groups.

When EMA-IgA testing cannot be performed, as in some centers in Türkiye, duodenal biopsy may be a safer approach to confirm the diagnosis, even when tTG-IgA levels are $\geq 10\times$ ULN, especially given the lifelong implications of starting a gluten-free diet. Our findings also support retaining a biopsy-based diagnostic approach in children with tTG-IgA levels $<10\times$ ULN.⁷

In the univariable logistic regression analysis, tTG-IgA $\geq 10\times$ ULN was not significantly associated with Marsh grade 3, whereas duodenal endoscopic abnormalities showed a strong, statistically significant association. Because only 11 patients did not have Marsh grade 3, a reliable multivariable model could not be constructed. The findings were, therefore, interpreted only at the univariable level and should be considered associative and hypothesis-generating. Nevertheless, this association suggests that endoscopic assessment may provide additional diagnostic value. The prevalence of the ultra-short CD pattern in our

Table 5. Univariable regression analysis for factors associated with Marsh grade 3		
Variable	Univariable OR (95% CI)	p
Age, years	1.123 (0.941-1.340)	0.199
Sex, female (ref: male)	1.946 (0.560-6.757)	0.295
Family history of celiac disease (ref: no)	1.431 (0.355-5.760)	0.614
BMI-for-age/WFH z-score	1.305 (0.776-2.194)	0.315
HFA z-score	1.318 (0.751-2.315)	0.336
Wasting (ref: no)	0.967 (0.196-4.777)	0.967
Stunting (ref: no)	0.426 (0.123-1.475)	0.178
Symptomatic (ref: asymptomatic)	3.389 (0.625-18.383)	0.157
Number of symptoms ≥ 3 (ref: <3)	1.859 (0.539-6.410)	0.326
tTG-IgA $\geq 10\times$ ULN	3.036 (0.863-10.684)	0.084
Any bulbar or distal duodenal endoscopic abnormality (ref: no)	11.810 (2.697-51.703)	0.001
Any additional non-celiac histopathological finding (ref: no)	0.434 (0.090-2.097)	0.299
Odds ratios and 95% confidence intervals were estimated using univariable binary logistic regression. BMI: Body mass index, CI: Confidence interval, HFA: Height-for-age, OR: Odds ratio, ref: Reference category, tTG-IgA: Tissue transglutaminase immunoglobulin A, ULN: Upper limit of normal, WFH: Weight-for-height		

cohort (5.0%; 7/141) is consistent with the rates of 2.5%-11.4% reported in pediatric series for cases in which histopathological involvement is confined to the duodenal bulb.²⁴⁻²⁶ This finding further emphasizes the importance of obtaining duodenal bulb biopsies during diagnostic evaluation.⁷ In accordance with expert recommendations, patients in our study with this pattern were started on a gluten-free diet.²⁷ Recent pediatric data indicate that ultra-short CD presents a milder serological profile than extensive CD. Demir et al.²⁸ reported significantly lower tTG-IgA levels in children with ultra-short CD, despite a similar prevalence of associated autoimmune diseases. In addition, among patients who underwent repeat endoscopy while maintaining a gluten-containing diet, villous atrophy remained confined to the duodenal bulb and did not progress to the distal duodenum. In our cohort, 3 of 7 patients with an ultra-short CD pattern (42.9%) had tTG-IgA levels $<10\times$ ULN. These findings suggest that reliance on high serological thresholds may miss some patients with ultra-short CD. Although current evidence does not specifically validate a no-biopsy algorithm for this phenotype, it nonetheless underscores the importance of obtaining duodenal bulb biopsies, particularly in children with lower tTG-IgA levels.

Study Limitations

This study has several limitations. Its retrospective, single-center design and small sample size limit the generalizability of the findings and the strength of subgroup analyses. The small number of patients without Marsh grade 3 (n=11) precluded

the development of a reliable multivariable regression model; therefore, only univariable associations were evaluated. A key strength is the direct comparison of the diagnostic performance of the tTG-IgA threshold against histopathological findings under real-world conditions in which access to EMA-IgA testing is limited. Our findings complement previous studies reporting limited applicability of the ESPGHAN no-biopsy diagnostic criteria in Türkiye.²²

CONCLUSION

At our institution, tTG-IgA $\geq 10 \times$ ULN showed a high PPV for Marsh grade 3 (94.4%; 95% CI, 88.4-97.4). However, because Marsh grade 3 was absent in 6 patients with high-titer tTG-IgA, this threshold should not be used as a standalone criterion for a no-biopsy diagnosis. When EMA-IgA testing is not locally available, sending a second serum sample to a reference laboratory is a reasonable alternative, consistent with current guidelines, and if this is not feasible, confirmation by duodenal biopsy may be safer.

Ethics

Ethics Committee Approval: The Harran University Non-Interventional Clinical Research Ethics Committee approved the study on April 14, 2026 (protocol no: HRÜ/26.01.01)

Informed Consent: Informed consent was not obtained because of the retrospective design of the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.T.D., G.A.H., Concept: D.T.D., G.A.H., A.B., Design: D.T.D., Data Collection or Processing: D.T.D., G.A.H., A.B., Analysis or Interpretation: D.T.D., Literature Search: D.T.D., G.A.H., Writing: D.T.D., G.A.H., A.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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