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ASSOCIATION BETWEEN CLINICAL FEATURES, IGA TISSUE TRANSGLUTAMINASE ANTIBODY, AND HISTOLOGICAL FINDINGS IN CELIAC DISEASE

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ABSTRACT

Background: Celiac disease (CD) is an immune-driven disorder of the intestine that develops in genetically predisposed individuals after consuming gluten. It manifests with diverse gastrointestinal and extra-intestinal features. Diagnosis relies on a combination of serology and histology, with IgA tissue transglutaminase (tTG-IgA) serving as a key biomarker and Marsh classification grading the severity of mucosal damage. However, limited data exist from Pakistan on the correlation between clinical features, serological markers, and histopathological changes.

Objective: To determine the frequency of clinical presentations, IgA tTG antibody levels, and histopathology findings in Celiac Disease patients, and to assess the associations between them.

Methods: We conducted a cross-sectional study in Department of Medicine of Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan during June 2025 and September 2025, after enrolling a total of 139 patients aged 14–60 years with positive IgA tTG using non-probability consecutive sampling. Clinical features were documented through history and examination, tTG-IgA levels were measured using ELISA, and duodenal biopsies were scored as per Marsh classification. Chi-square/Fisher's exact tests were applied for associations using SPSS 27.

Results: Median age of the participants was 35 years, with 54.7% males. Impaired growth (36%), chronic diarrhea (33.1%), muscle wasting (21.6%), and anemia (34.5%) were the most frequent clinical features. Tissue transglutaminase IgA was positive in 91.4% of patients, with 41% weak positive, 48.9% moderate positive, and 1.4% strong positive. Histologically, most patients exhibited Marsh I–III changes. No significant association was observed between Marsh classification and clinical features, whereas a strong correlation was found between Marsh stage and both tTG-IgA positivity and antibody levels ($p < 0.01$).

Conclusion: CD in our population presents with varied clinical and nutritional features, but histological severity is more closely linked with serological markers than with symptoms. These findings highlight the diagnostic value of tTG-IgA in predicting mucosal damage and emphasize the need for integrated serological and histological assessment in clinical practice.

KEYWORDS: Celiac disease, Clinical features, Marsh classification, Tissue transglutaminase IgA (tTG-IgA)

INTRODUCTION

Celiac disease (CD) is an autoimmune chronic disease caused by ingestion of gluten in people who are genetically predisposed. This condition results in inflammation and damage to the small intestine, which affects nutrient absorption and results in a spectrum of clinical symptoms. It is estimated that around 1% of the global population is affected, but many patients remain undiagnosed or misdiagnosed because clinical presentation can vary widely(1). Celiac disease, often perceived as a gastrointestinal disorder with diarrhea and malabsorption, may have extra-intestinal presentations including iron-deficiency anemia, osteoporosis, neurological symptoms and skin lesions (e.g., dermatitis herpetiformis)(2).

The most frequently employed biomarker for diagnosis of celiac disease is IgA tissue transglutaminase (tTG) antibodies. Elevated levels of IgA tTG antibodies are highly specific for celiac disease and have a sensitivity of

around 90% to 98%, making them a reliable screening tool(3). Histological examination remains the gold standard for confirming celiac disease. The **Marsh classification** is used to assess the severity of intestinal damage, which ranges from mild changes (Marsh 1) to complete villous atrophy (Marsh 3c). Studies have shown a significant correlation between IgA tTG antibody levels and histological findings, with higher levels typically associated with more severe histological damage (Marsh 3)(4). A study compared clinical features across age groups. The most prominent clinical features were chronic diarrhea in adolescents while mostly psychiatric issues in adults, greatly varying within different age groups(5). In a study investigating the epidemiological and clinical characteristics of celiac disease in China, among the 2884 tested, 50 (0.17%) had duodenal and ileal villous atrophy. Chronic diarrhea, anorexia, anemia and lower body mass index were the common clinical presentations found. Higher detection rates were found in Kazakh and Uyghurs populations(6). A study investigating celiac disease in 150 diagnosed patients in Iran found a slight female predominance (52%). Abdominal pain (38%), chronic diarrhea (32%), and nausea (30%), with 30% exhibiting iron-deficiency anemia were common clinical features. IgA anti-TTG antibodies were positive in 96% of the patients while Biopsy results revealed Marsh I, II, and III histological changes in 76%, 10%, and 52% of patients, respectively(7). Another study showed that mild endoscopic damage significantly correlated with Marsh II-IIIa classification ($P < 0.005$), while severe damage was found with Marsh IIIb-IIIc ($P < 0.0005$). Mild endoscopic findings ($P < 0.001$), were found in younger patients whereas older patients showed severe damage ($P < 0.005$). More endoscopic damage was found in Classical celiac disease compared to subclinical/silent forms ($P < 0.001$)(8) . Celiac disease (CeD), once considered confined to individuals of European origin, is now recognized as a disorder globally affecting approximately 1% of the population of the world, including non-Caucasian groups in Africa and Asia. Rising prevalence and incidence have been reported in Europe and the United States, largely attributable to improved awareness and the availability of reliable serological tests that have simplified diagnosis. While previously regarded as a childhood condition restricted to small intestinal pathology, Celiac Disease is now understood to affect all age groups and to represent a systemic disease involving multiple organs such as the skin, liver, bones, and brain. Consequently, its clinical spectrum encompasses both gastrointestinal and extra-gastrointestinal manifestation(9)**.** A comparative study from a large Celiac Clinic cohort (n=473) examined age-related differences in the clinical features of celiac disease across children, adolescents, and adults. The findings demonstrated that while gastrointestinal (GI) manifestations were common in all groups, they were significantly more prevalent in adults (81.9%) compared to children (66.4%) and adolescents (59.8%) (PR 1.167; 95% CI: 1.094–1.244). Similarly, non-intestinal manifestations were observed more frequently in adults (94.8%) than in children (75.6%) and adolescents (87.4%) (PR 1.112; 95% CI: 1.060–1.168). Symptom patterns also differed by age, with abdominal discomfort predominating in younger patients, while psychiatric problems were most common among adults. These results highlight important clinical variations in disease presentation across age groups, underscoring the need for age-tailored diagnostic and management approaches(10)**.** A study from the Veneto region evaluated socio-demographic, clinical, and psychological profiles of 110 newly diagnosed adult celiac disease (CeD) patients (81% females; mean age 37.5 years). Patients were classified as classical (51%), nonclassical (45%), or asymptomatic (4%). Classical CeD was associated with lower quality of life (QoL), whereas nonclassical patients reported higher levels of depression. Diagnostic delay exceeding seven months was observed in over 60% of symptomatic patients, and prolonged gastrointestinal symptoms were significantly linked with poorer scores in physical health, social functioning, and general health domains of the SF-36. Female patients reported lower overall QoL. These findings emphasize the impact of presentation type, diagnostic delay, and sex on QoL and psychological wellbeing in CeD(11)**.** A recent retrospective study of 134 newly diagnosed adult celiac disease (CD) patients (median age 35 years; 78% female) demonstrated that gastrointestinal (GI) symptoms, such as diarrhea, abdominal bloating, and dyspepsia, remained the leading presentation, reported by 59% of patients. Extra-intestinal manifestations (EIMs) including iron deficiency anemia, infertility, dermatitis, osteoporosis, and elevated transaminase levels accounted for 30% of initial presentations, while 11% of patients were asymptomatic, diagnosed due to family history or concomitant autoimmune thyroid disease. Notably, 20 of the 79 patients with GI symptoms did not achieve complete resolution after a gluten-free diet (GFD), with most non-responsive cases attributable to overlap with irritable bowel syndrome (IBS) or, less commonly, lactose intolerance. These findings reinforce the need for clinicians to consider both EIMs and coexisting functional disorders in the diagnostic and therapeutic approach to CD(12). In Pakistan, where the true prevalence of celiac disease (CD) remains uncertain due to underdiagnosis and limited awareness, recent data provide important insights into clinical presentation. A cross-sectional study conducted at the Jinnah Postgraduate Medical Center, Karachi, evaluated 142 adult CD patients (mean age 23 ± 6 years; 62.7% female). The majority (91.5%) presented with classical CD (CCD), while 25% had non-classical CD (NCCD). Nutritional deficiencies, including anemia, vitamin B12 and folate deficiency, osteopenia, and low body mass index, were significantly more frequent in the CCD group. Hypothyroidism and polycystic ovarian syndrome (PCOS) were the most common associated comorbidities. These findings highlight the diverse manifestations of CD in Pakistani adults and emphasize the need for greater clinical suspicion, particularly in patients presenting with unexplained extra-intestinal features such as anemia and bone pain(13). In pediatric populations, celiac disease (CD) also demonstrates diverse clinical manifestations. A cross-sectional study conducted at the Department of Pediatrics, DHQ Hospital, Faisalabad, assessed 90 children aged 1–14 years diagnosed with CD. The most common presenting features included chronic diarrhea (47.8%), iron deficiency anemia (43.3%), failure to thrive (32.2%),

short stature (26.7%), and dermatitis herpetiformis (24.4%). These findings confirm that diarrhea, anemia, and growth failure remain the predominant presentations of CD in children within Pakistan(14).
Studies have explored the relationship between serological and histopathological findings, with many suggesting that elevated IgA tTG antibody levels may correlate with more advanced histological stages of celiac disease, however there is a considerable literature gap regarding the associations between clinical, serological and histological features especially in Pakistan. Our study aims to shed light on the possible correlation between the clinical, serological and histological characteristics of Celiac Disease which might help clinicals in evaluating the management and gain insights into the disease pattern of Celiac disease.

Objectives
Our objective was to determine the frequency of clinical presentations, IgA tissue transglutaminase (tTG-IgA) antibodies, and histological findings in patients with celiac disease and to evaluate the association between clinical features, tTG-IgA levels, and histopathological changes based on Marsh classification.

Operational Definitions
1. **Celiac Disease:** Defined as a patient with positive IgA tTG (>10 U/mL).
2. **Marsh Classification:** Histopathological findings on duodenal biopsy were graded as:
- Marsh 0: Normal mucosal lining.
- Marsh 1: Raised intraepithelial lymphocytes with normal villous architecture.
- Marsh 2: Raised intraepithelial lymphocytes with crypt hyperplasia.
- Marsh 3a: Partial villous atrophy.
- Marsh 3b: Subtotal villous atrophy.
- Marsh 3c: complete atrophy of the mucosal villa.
3. **Clinical Features:** Defined as the following:
- Impaired growth: BMI < 17.
- Chronic diarrhea: ≥3 episodes of loose stools persisting for >2 weeks.
- Muscle wasting: Loss of muscle mass identified on clinical examination.
- Anemia: Hemoglobin < 12 g/dl.
4. **tTG-IgA Levels:** Levels >10 U/mL were considered positive and categorized as:
- Weak positive: 10–30 U/mL.
- Moderate positive: 30–100 U/mL.
- Strong positive: >100 U/mL.

METHODOLOGY

Study Design and Setting
This cross-sectional study was conducted at the Department of Medicine, Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan from June 2025 to September 2025, after approval from the institutional ethical review board. Using the OpenEpi sample size calculator, and assuming an anticipated frequency of Marsh II changes in celiac disease patients as 10%(7), a confidence limit of 5%, and a 95% confidence level, the required sample size was calculated to be 139. Participants were enrolled through non-probability consecutive sampling.
Inclusion Criteria
- Patients diagnosed with celiac disease based on positive IgA tTG.
- Both male and female patients.
- Age range: 14–60 years.

Exclusion Criteria
- Patients already on a gluten-free diet.
- Patients with IgA deficiency.
- Patients with other gastrointestinal disorders such as Crohn's disease, ulcerative colitis, or Hirschsprung's disease.

Data Collection Procedure
After obtaining ethical approval, informed written consent was taken from all participants. A structured questionnaire was used to collect demographic details (age, gender, weight, height, BMI, and duration since diagnosis). Clinical features, including impaired growth, chronic diarrhea, muscle wasting, and anemia, were assessed through history and physical examination. Serum tTG-IgA levels were measured using enzyme-linked immunosorbent assay (ELISA). Duodenal biopsies were performed and evaluated according to Marsh classification.
Data Analysis
Data were analyzed using Statistical Package for Social Sciences (SPSS) version 27. Descriptive statistics were applied: quantitative variables (age, weight, height, BMI) were summarized using mean, median, standard deviation, and interquartile range, while qualitative variables (gender, clinical features, Marsh classification, and serological findings) were expressed as frequencies and percentages. Associations between clinical features,

serology, and histology were assessed using Chi-square or Fisher's exact test, with a p-value < 0.05 considered statistically significant.

RESULTS

The median age of participants was 35 years, with an interquartile range (IQR) of 26 years. The median weight was 62 kg (IQR: 29), and the median height was 1.63 meters (IQR: 0.26). The median body mass index (BMI) was 21.9 (IQR: 10.7). The median tissue transglutaminase IgA (tTG-IgA) level was 30.3 (IQR: 22.10). All variables were tested for normality using the Shapiro-Wilk test and were found to be non-normally distributed ($p < 0.01$). (**Table 1**)

Table 1 Demographic data of the patients

characteristics	Median	Interquartile Range	P-value (Shapiro wilk)
age of the participant (in years)	35	26	<0.01
weight of the participant (kgs)	62	29	<0.01
Height of participant (in meters)	1.63	0.26	<0.01
BMI of the Participant	21.9	10.7	<0.01
tTg-IgA value	30.3	22.10	<0.01

Out of 139 participants, 76 (54.7%) were male and 63 (45.3%) were female. In terms of BMI categories, 42 patients (30.2%) were underweight, 48 (34.5%) had normal weight, 18 (12.9%) were overweight, and 31 (22.3%) were obese. Impaired growth was observed in 50 patients (36.0%), while 89 (64.0%) showed no growth impairment. Chronic diarrhea was present in 46 participants (33.1%), whereas 93 (66.9%) did not report this symptom. Muscle wasting was observed in 30 patients (21.6%) and absent in 109 (78.4%). Iron deficiency anemia was found in 48 patients (34.5%), with the remaining 91 (65.5%) not affected. Regarding serological markers, tissue transglutaminase IgA was positive in 127 patients (91.4%) and negative in 12 (8.6%). Among tTG-IgA levels, 12 (8.6%) were negative, 57 (41.0%) weak positive, 68 (48.9%) moderate positive, and 2 (1.4%) strong positive. Histologically, based on Marsh classification, 12 (8.6%) were Marsh 0, 42 (30.2%) Marsh 1, 45 (32.4%) Marsh 2, 38 (27.3%) Marsh 3a, 2 (1.4%) Marsh 3b, and 12 (8.6%) Marsh 3c. (**Table 2**)

Table 2 Gender, BMI and clinical features

Cllinical Features		Frequency (n=139)	Percentages (%)
Gender	Male	76	54.7
	Female	63	45.3
BMI	Underweight	42	30.2
	Normal Weight	48	34.5
	Overweight	18	12.9
	Obese	31	22.3
Impaired Growth	Yes	50	36.0
	No	89	64.0
Chronic Diarrhea	Yes	46	33.1
	No	93	66.9
Muscle Wasting	Yes	30	21.6
	No	109	78.4
Iron Deficiency Anemia	Yes	48	34.5
	No	91	65.5
Tissue TG-IgA	Positive	127	91.4
	Negative	12	8.6
tTG-IGA Levels	Negative	12	8.6
	Weak Positive	57	41.0
	Moderate Positive	68	48.9
	Strong Positive	2	1.4
MARSH Classification	MARSH 0	12	8.6
	MARSH 1	42	30.2
	MARSH 2	45	32.4
	MARSH 3a	38	27.3
	MARSH 3b	2	1.4
	MARSH3c	12	8.6

Chi-square and Fisher's Exact tests were applied to assess the association between Marsh classification and clinical features. No significant associations were observed between Marsh stages and impaired growth ($p=0.706$), chronic diarrhea ($p=0.235$), muscle wasting ($p=0.677$), or iron deficiency anemia ($p=0.065$). However, a highly

significant association was found between Marsh classification and tissue transglutaminase IgA positivity ($p < 0.01$), as all patients with positive tTG-IgA showed Marsh stage changes, while those negative for tTG-IgA were only in Marsh 0. Similarly, Marsh classification was strongly associated with different levels of tTG-IgA ($p < 0.01$): patients with weak positivity clustered in Marsh 1, those with moderate positivity in Marsh 2 and 3a, and strong positivity in Marsh 3c. This indicates that increasing histological severity (Marsh stage) corresponded with higher tTG-IgA positivity and levels, highlighting a strong correlation between serological markers and histological findings. (**Table 3**)

Table 3 chi-square/ Fischer's Exact test between Marsh Histological features and clinical features

		Marsh Classification						
		Marsh 0	Marsh 1	Marsh 2	Marsh 3a	Marsh 3b	Total	Chi Square /Fischer Exact p-value
Impaired growth	yes	6	14	16	14	0	50	0.706
	no	6	28	29	24	2	89	
	Total	12	42	45	38	2	139	
Chronic Diarrhea	yes	3	12	14	15	2	46	0.235
	no	9	30	31	23	0	93	
	Total	12	42	45	38	2	139	
Muscle wasting	yes	1	10	10	8	1		0.677
	no	11	32	35	30	1		
	Total	12	42	45	38	2	139	
Iron Deficiency anemia	yes	3	13	12	18	2	48	0.065
	no	9	29	33	20	0	91	
	Total	12	42	45	38	2	139	
Tissue TG-IgA	Positive	0	42	45	38	2	127	< 0.01
	Negative	12	0	0	0	0	12	
	Total	12	42	45	38	2	139	
tTG-IGA Levels	Negative	12	0	0	0	0	12	< 0.01
	Weak Positive	0	42	15	0	0	57	
	Moderate Positive	0	0	30	38	0	68	
	Strong Positive	0	0	0	0	2	2	
	Total	12	42	45	38	2	139	

DISCUSSION

In the present study, the median age of participants was 35 years, with males comprising a slight majority. A substantial proportion of patients were underweight or obese, reflecting nutritional imbalances in celiac disease. Common clinical manifestations included impaired growth, chronic diarrhea, muscle wasting, and iron deficiency anemia. Serologically, the majority of patients were positive for tissue transglutaminase IgA, with varying degrees of antibody levels. Histologically, most patients demonstrated changes consistent with Marsh grades 1–3, with relatively few in Marsh 0. Importantly, while no significant associations were found between Marsh classification and clinical features such as growth impairment, diarrhea, or anemia, a strong correlation was observed between histological severity and serological markers. Higher Marsh grades were significantly associated with both positivity and increasing levels of tTG-IgA, indicating that antibody titers reliably reflect the degree of mucosal damage.

In comparison with our findings, In Pakistan, several studies have highlighted the clinical and histopathological spectrum of celiac disease. A study from Sindh reported that the majority of pediatric patients presented with a typical clinical picture of celiac disease, with chronic diarrhea being the most common manifestation(15). Similarly, a study from Rawalpindi assessed celiac disease using Marsh classification in children aged 1–14 years and found that over half (57.7%) of the cases were histology-positive, with Marsh IIIa being the predominant subtype. While the disease appeared more frequent in males, the gender difference was not statistically significant. Importantly, the authors emphasized that celiac disease should be considered as a potential cause of unexplained anemia, warranting histopathological evaluation when suspected(16). Extending these findings to adults, a study from Peshawar examined 50 small intestinal biopsies and demonstrated that males in the age group of 21–30 years were the most affected. Partial villous atrophy was the predominant histological feature, while intraepithelial lymphocytosis was universally observed. Notably, immunohistochemistry with CD3 and CD20 markers corroborated the reliability of conventional histology for lymphocyte assessment. These findings reinforce the role of biopsy-based evaluation, supported by immunohistochemistry, in diagnosing adult celiac disease and

highlight partial villous atrophy as the most frequent lesion in Pakistani patients(17)**.** Finally, a study comparing endoscopic findings with serological testing demonstrated that while anti-tTG antibodies had strong diagnostic utility, duodenal endoscopic findings alone lacked sensitivity, with nearly half of seropositive patients showing normal mucosa despite underlying villous atrophy. The majority of these patients were young females, further illustrating demographic differences between adult and pediatric cases(18).

CONCLUSION

Our study concluded that celiac disease may present with diverse clinical, serological, and histological profiles in our population. While we observed common clinical features such as growth impairment, chronic diarrhea, muscle wasting and anemia, their association with histological severity was not statistically significant. Instead, a strong association was evident between Marsh classification and serological markers, with higher tTG-IgA titers corresponding to more advanced mucosal damage. These findings reinforce the diagnostic value of serology, particularly tTG-IgA, as a reliable predictor of histological severity.

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