

## Original Article



# Diagnostic Value of Immunoglobulin G Anti-Deamidated Gliadin Peptide Antibody for Diagnosis of Pediatric Celiac Disease: A Study from Shiraz, Iran

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## ABSTRACT

**Purpose:** Screening serologic tests are important tools for the diagnosis of celiac disease (CD). Immunoglobulin (Ig)G anti-deamidated gliadin peptide (anti-DGP) is a relatively new autoantibody thought to have good diagnostic accuracy, comparable to that of anti-tissue transglutaminase (anti-tTG) antibody.

**Methods:** Pediatric patients (n=86) with a clinical suspicion of CD were included. Duodenal biopsy, anti-tTG, and IgG anti-DGP antibody tests were performed. The patients were divided into CD and control groups based on the pathological evaluation of duodenal biopsies. The diagnostic accuracy of serological tests was determined.

**Results:** IgA anti-tTG and IgG anti-DGP antibodies were positive in 86.3% and 95.4% of patients, respectively. The sensitivity, specificity, and diagnostic accuracy of the IgA anti-tTG test were 86.3%, 50.0%, and 68.6%, respectively, and those of the IgG anti-DGP test were 95.4%, 85.7%, and 90.7%, respectively. The area under the receiver operating characteristic (ROC) curve was 0.84 (95% confidence interval [CI], 0.74–0.91) for IgA anti-tTG test and 0.93 (95% CI, 0.86–0.97) for IgG anti-DGP test. The comparison of IgA anti-tTG and IgG anti-DGP ROC curves showed a higher sensitivity and specificity of the IgG anti-DGP test.

**Conclusion:** IgG anti-DGP is a reliable serological test for CD diagnosis in children. High tTG and DGP titers in the serum are suggestive of severe duodenal atrophy. The combined use of IgA anti-tTG and IgG anti-DGP tests for the initial screening of CD can improve diagnostic sensitivity.

**Keywords:** Celiac disease; Serology; Anti-tissue transglutaminase antibody; Gliadin peptide; Perdiatrics

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**Conflict of Interest**

The authors have no financial conflicts of interest.

**INTRODUCTION**

Celiac disease (CD) is a common, gluten-sensitive, chronic immune-mediated disorder with a prevalence rate of approximately 1% in both adults and children. The prevalence of CD has increased significantly over the past decades. The clinical spectrum of CD is broad, including typical intestinal findings (e.g., chronic diarrhea, abdominal pain, and weight loss) and atypical extraintestinal findings (e.g., anemia, hypertransaminasemia, enamel dysplasia, and neurological disturbances). High-risk individuals are detected by screening serologic tests, and duodenal biopsy during upper endoscopy is the gold standard for diagnosis [1-5].

The presence of highly specific autoantibodies is characteristic of CD. The two highly accurate autoantibodies used to diagnose CD are anti-tissue transglutaminase (anti-tTG) and anti-endomysial antibodies (anti-EMA) [4-6]. Recently, a third antibody against deamidated gliadin peptide (DGP) was introduced and is believed to be specific to active CD. In the recent years, the sensitivity and specificity of anti-DGP antibodies have been studied, and it has been concluded that their diagnostic value is similar to that of immunoglobulin (Ig)A anti-tTG, and they may even be preferred for very young children [4-9]. However, the usefulness of isolated positive anti-DGP tests has not yet been confirmed in the pediatric population [10].

This study aimed to analyze the diagnostic performance of anti-DGP IgG and anti-tTG IgA for CD in children. We also determined the best cutoff values for both serologic tests with the highest sensitivity and specificity by using receiver operating characteristic (ROC) curves.

**MATERIALS AND METHODS****Patients**

This cross-sectional study was conducted from 2019 to 2020 in outpatient pediatric gastroenterology clinics linked to Shiraz University of Medical Sciences. This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1398.009).

Gastrointestinal symptoms, such as diarrhea, recurrent abdominal pain, failure to thrive, dyspepsia, constipation, bloating, nausea, and vomiting or extraintestinal signs such as, iron-deficiency anemia, hypertransaminasemia of unknown origin, short stature, aphthous stomatitis, unexplained osteoporosis, and dental enamel defects were evaluated by gastroenterologists. Each patient was suspected of having CD according to clinical manifestations and laboratory findings and was referred for upper gastrointestinal tract endoscopic evaluation with duodenal biopsy. At the same time, adequate amount blood was collected from all patients for serologic tests, including IgG anti-DGP and IgA anti-tTG antibodies. The patients or their parents provided signed informed consent according to the Shiraz University of Medical Sciences guidelines.

Consecutively, we enrolled 86 pediatric patients (aged <18 years) from 2019 to 2020. None of the patients were IgA-deficient. Patients on a gluten-free diet were also excluded. The patients were classified into two groups: the CD group and the non-CD group (control group), according to the pathological evaluation of duodenal biopsy.

### IgG anti-DGP test

All serum samples were kept frozen at  $-70^{\circ}\text{C}$  until analysis for serological tests. Anti-DGP IgG was measured in the sera of patients using an enzyme-linked immunosorbent assay (ELISA) kit (DiaMetra, Perugia, Italy). According to the manufacturer's instructions, the cutoff values were  $<15$ ,  $15\text{--}30$ , and  $>30$  AU/mL as negative, equivocal, and positive, respectively.

### IgA anti-tTG test

IgA anti-tTG was measured in the sera of the patients using an ELISA kit (AESKULISA tTG-A New Generation; AESKU.DIAGNOSTICS, Wendelsheim, Germany). According to the manufacturer's instructions, the cutoff values were  $<12$ ,  $12\text{--}18$ , and  $>18$  U/mL as negative, equivocal, and positive, respectively.

### Histopathologic examination of duodenal biopsy

Multiple biopsy samples were obtained from the second and first portions of the duodenum during upper gastrointestinal tract endoscopy. The samples were sent to the pathology laboratory in separate bottles. It was mandatory to perform four biopsies from the distal duodenum and two biopsies from the duodenal bulb. The biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Duodenal biopsies were carefully reviewed by a gastrointestinal pathologist (MHA) and evaluated according to the Marsh–Oberhuber classification. CD was defined as Marsh grade 3 (total villous atrophy, subtotal villous atrophy, or partial villous atrophy). Individuals with Marsh scores of 0, 1, and 2 were considered to have no CD.

### Statistical analysis

All statistical analyses were performed using SPSS version 24 (IBM Co., Armonk, NY, USA). An independent *t*-test was used to compare the distributions of anti-DGP and anti-tTG antibodies and the mean ages between patients and controls. The cutoff values for IgG anti-DGP and IgA anti-tTG antibody reactivity with the highest sensitivity and specificity were determined using ROC curves. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using both the manufacturer's values and the optimal cut-off points obtained based on the ROC-associated Youden index. Analysis of variance was used to assess the correlation between antibody levels and the severity of villous atrophy of the duodenal mucosa. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### Patients' data

In this study, we evaluated 86 pediatric patients with clinical suspicion of CD (male [M]/female [F]: 40/46, mean age: 8 years, standard deviation [SD]: 3.5 years). According to duodenal biopsy, the patients were categorized into the CD group (44 patients, M/F: 18/26, mean age: 9 years, SD: 3.2 years) and control group (42 patients, M/F: 22/20, mean age: 7 years, SD: 3.6 years). Histopathological examination of duodenal biopsies in the CD group showed Marsh grade 3a in 16 (36.3%), Marsh grade 3b in 22 (50%), and Marsh grade 3c in six (13.7%) patients. Thirty (68.2%) patients showed histological evidence of CD in both parts of the duodenum, 10 (22.7%) only in the bulb, and four (9.1%) only in the second portion of the duodenum.

### Serological tests

IgA anti-tTG antibody was positive in 86.3% of CD patients according to the manufacturer's cutoff values, while IgG anti-DGP antibody was positive in 95.4% of CD patients, according to the optimal ROC cutoff values. **Table 1** shows the serological test values in the CD and control groups. The mean±SD titer of IgA anti-tTG was 226.2±178.7 U/mL (median: 222.5, range 6.6–440) and that of IgG anti-DGP was 78.9±80.2 AU/mL (median: 48, range 8–280) in the CD group. The mean±SD titer of IgA anti-tTG was 36.9±48.7 U/mL (median: 16.75, range 0.2–180) and that of IgG anti-DGP was 11.4±17.5 AU/mL (median: 8, range 2–116) in the control group. The levels of IgA anti-tTG and IgG anti-DGP antibodies were significantly higher in patients with CD than in controls ( $p<0.00001$ ). Moreover, the levels of IgA anti-tTG and IgG anti-DGP antibodies correlated with the severity of villous atrophy in duodenal biopsies ( $p<0.00001$ ) (**Figs. 1 and 2**). The IgA anti-tTG and IgG anti-DGP tests had false-negative results in six and two patients (if we consider equivocal results as negative), and false-positive results in 21 and 6 patients, respectively.

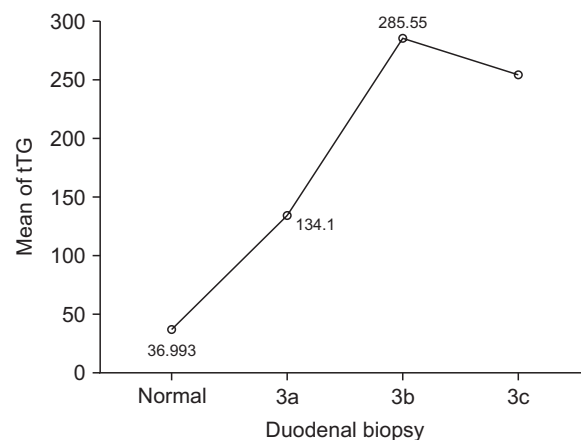
ROC curves were used to analyze the sensitivity and specificity of the tests and to determine the optimal cutoff values for seropositivity. The area under the curve was 0.84 (95% confidence interval [CI], 0.74–0.91) for IgA anti-tTG test (**Fig. 3**) and 0.93 (95% CI, 0.86–0.97) for IgG anti-DGP test (**Fig. 4**). Based on the ROC curves, the optimal cutoff value for IgA anti-tTG positivity was 180 U/mL with a sensitivity of 54.5%, specificity of 100%, and likelihood ratio of 22.9; the optimal cutoff value for IgG anti-DGP positivity was 11.8 AU/mL with a sensitivity of 95.4%, specificity of 85.7%, and likelihood ratio of 6.6. The comparison of IgA anti-tTG and IgG anti-DGP ROC curves showed a higher sensitivity and specificity of the IgG anti-DGP test ( $p=0.02$ ).

**Table 1.** IgA anti-tTG and IgG anti-DGP test results in the CD and control groups

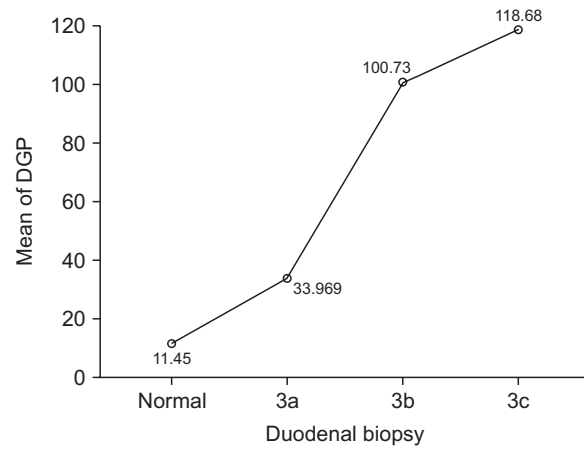
Serological test	CD group			Control group	Total
	Marsh 3a	Marsh 3b	Marsh 3c		
IgA anti-tTG (manufacturer cutoff value)					
Negative or equivocal (<18)	5 (5.8)	1 (1.2)	0 (0)	21 (24.4)	27 (31.4)
Positive (>18)	11 (12.8)	21 (24.4)	6 (7.0)	21 (24.4)	59 (68.6)
Total	16 (18.6)	22 (25.6)	6 (7.0)	42 (48.8)	86 (100)
IgG anti-DGP (optimal ROC cutoff value)					
Negative (<11.8)	2 (2.3)	0 (0)	0 (0)	36 (41.9)	38 (44.2)
Positive (>11.8)	14 (16.3)	22 (25.6)	6 (7.0)	6 (7.0)	48 (55.8)
Total	16 (18.6)	22 (25.6)	6 (7.0)	42 (48.8)	86 (100)

Values are presented as number (%).

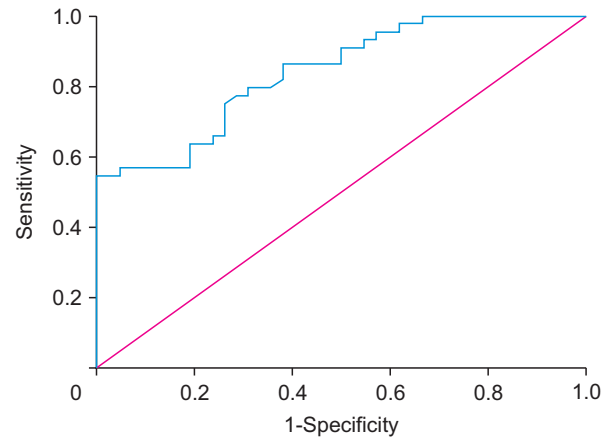
Ig: immunoglobulin, tTG: tissue transglutaminase, DGP: deamidated gliadin peptide, CD: celiac disease, ROC: receiver operating characteristic.



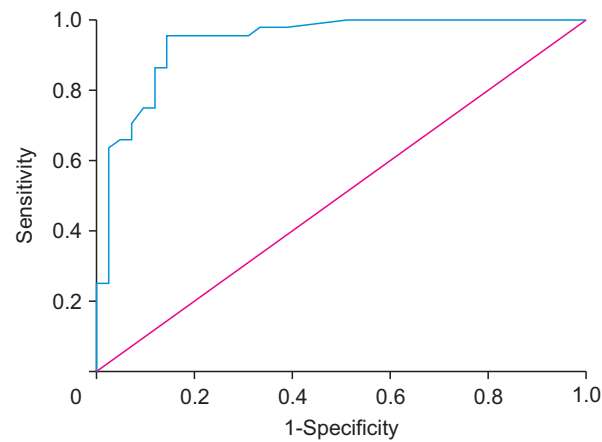
**Fig. 1.** Immunoglobulin A anti-tissue transglutaminase (tTG) level correlates with the severity of villous atrophy in duodenal biopsies.



**Fig. 2.** Immunoglobulin G anti-deamidated gliadin peptide (DGP) level correlates with the severity of villous atrophy in duodenal biopsies.



**Fig. 3.** Receiver operating characteristics of immunoglobulin A anti-tissue transglutaminase in all the children: area under the curve=0.84 (95% confidence Interval, 0.74–0.91).



**Fig. 4.** Receiver operating characteristics of immunoglobulin G anti-deamidated gliadin peptide in all the children: area under the curve=0.93 (95% confidence interval, 0.86–0.97).

## Diagnostic Value of IgG DGP in Patients with CD

**Table 2.** Diagnostic performance of IgA anti-tTG and IgG anti-DGP tests based on the manufacturer and ROC optimal cutoff values

Serological test	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC (%)
IgA anti-tTG	18 (manufacturer)	86.3	50	64.4	77.7	68.6	
	180 (ROC optimal)	54.5	100	100	67.7	76.7	84.1
IgG anti-DGP	11.8 (ROC optimal)	95.4	85.7	87.5	94.7	90.7	93.7
	30 (manufacturer)	63.6	97.6	96.5	71.9	80.2	
Combination of both serologic tests	18 for tTG and/or 11.8 for DGP	100	45.2	65.6	100	73.2	

Ig: immunoglobulin, tTG: tissue transglutaminase, DGP: deamidated gliadin peptide, ROC: receiver operating characteristic, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve.

**Table 2** shows the sensitivity, specificity, PPV, and NPV of the serologic tests based on the manufacturer and ROC optimal cutoff values. In 30 patients with moderately increased IgA anti-tTG level (level 18–99 U/mL), the sensitivity, specificity, PPV, NPV and accuracy of IgG anti-DGP test were 92.3%, 82.3%, 80%, 93.3%, and 86.6% with the ROC optimal cutoff value, respectively. In patients with false-positive results for IgA anti-tTG, the NPV of the IgG anti-DGP test was 80.9% with the ROC curve optimal cutoff value. Eight patients had negative IgA anti-tTG and positive IgG anti-DGP (isolated positive DGP) results that showed a PPV of 75% for the DGP test. However, this PPV should be interpreted cautiously because of the small sample size. The combination of these two serological tests led to an improvement in sensitivity and NPV (both 100%) (**Table 2**).

There were 13 CD patients with focal involvement in duodenal biopsy evaluation, and the mean IgA anti-tTG and IgG anti-DGP value was 66.8 U/mL and 31.9 AU/mL, respectively ( $p=0.00008$  and  $p=0.006$ ).

## DISCUSSION

This cross-sectional study included 86 patients with clinical suspicion of CD who underwent endoscopic duodenal biopsy and serological tests for IgA anti-tTG and IgG anti-DGP. The patients were classified into CD and control groups, and the diagnostic accuracy of these tests was determined according to the manufacturer-suggested and ROC curve optimal cutoff values. The diagnostic accuracy of the IgG anti-DGP test was 90.7% according to the ROC optimal cutoff value. IgG anti-DGP antibody was superior to IgA anti-tTG in our pediatric population. The combination of these two serological tests resulted in a sensitivity of 100%.

The diagnostic accuracy of serological tests in CD has been evaluated in many previous studies [4,6-8,10-17]. DGP antibodies were recognized in 2001 after the identification of the pathophysiology of deamidation of gluten peptides by transglutaminase 2 in CD [18]. Both IgA and IgG anti-DGP tests are available; however, most studies have shown that the IgA anti-DGP test has lower sensitivity than the IgG anti-DGP test. Therefore, the IgG anti-DGP serological test is most commonly used in clinical practice [7,9,14]. IgG anti-DGP primarily exhibited favorable results as a screening test, and early studies reported diagnostic accuracy comparable to tTG, and some studies showed better test characteristics in patients <2 years of age [7-9,16,18]. Despite these promising findings, the results have not been consistent throughout the literature [13, 19]. While the sensitivity and specificity of IgG anti-DGP was reported to be more than 90% in some studies [4,8,9,11,17,19-21], other studies have reported lower sensitivity value [7,15,16,22,23]. It is important to note that the previous studies had some differences in study design and test methods such as age of study population, the use of manufacturer-suggested or ROC curve optimal cutoff values, and ELISA method in contrast

to other methods. Some differences in the diagnostic accuracies of serological tests may be related to these differences. In addition, some studies have evaluated the total anti-DGP antibody [12,13], similar to Olen et al. [13], who reported relatively poor test performance of DGP. In our study, the sensitivity, specificity, and accuracy of the IgG anti-DGP test in the pediatric population, using ELISA and ROC optimal cutoff values, were 95.4%, 85.7%, and 90.7%, respectively.

In contrast to previous studies [4,7,8,13,14], the sensitivity of the IgA anti-tTG test for CD was lower than that of the IgG anti-DGP test. The sensitivity of our test was 86.3% with an NPV of 77.7%. There were six false-negative results, four of them showed focal involvement in duodenal biopsy evaluation, and the IgG anti-DGP test was positive in all the six patients. The reason for this lower diagnostic accuracy of the tTG test is unclear but is likely due to the lack of standardization of commercial kits and therefore, we emphasize the need for quality management in celiac serological kits. This low accuracy could also be attributed to the relatively small sample size.

The concurrent use of IgA anti-tTG and IgG anti-DGP serological tests as screening for CD is controversial. There are some reports that opposed combination use [13,20,22,24] and others that are concordant with it [7,21]. The recent European Society for Pediatric Gastroenterology, Hepatology and Nutrition guidelines for CD recommend that adding an IgG anti-DGP test to IgA anti-tTG seldom improves sensitivity, so they advise a combination of total IgA and IgA anti-tTG as initial screening tests for CD [24]. In contrast, Oyaert et al. [21] showed that combining IgA anti-tTG and IgG anti-DGP and considering their antibody levels improved the serological diagnosis of CD. Our results indicate that the concurrent use of IgA anti-tTG and IgG anti-DGP can improve sensitivity and NPV. Therefore, performing both serologic tests for all suspected patients provides the most information, but is also most expensive. Therefore, this combination can be used in patients with IgA deficiency and in highly clinically suspected patients with negative tTG [21]. Additionally, some researchers believe that the IgG anti-DGP test is a good tool for diagnosing CD in patients aged <2 years of age [4,7,14], and it can replace IgA anti-EMA to confirm CD [7,25]. In a review by Catassi et al. [19], they concluded that IgA anti-tTG is the best CD screening test in children <2 years of age; however, the addition of IgG anti-DGP may increase the diagnostic sensitivity.

Many studies, including ours, have confirmed the relationship between the degree of duodenal atrophy and increased levels of tTG or DGP antibodies in the serum [4,7,9,11,14,21].

Evaluation of the diagnostic accuracy of the IgG anti-DGP test in pediatric patients with mildly to moderately increased IgA anti-tTG levels showed acceptable results (sensitivity and specificity of 92.3% and 82.3%, respectively) in our study; however, in the study by Dickerson et al. [12], the sensitivity and specificity were 86.7% and 56.3%, respectively.

Two previous studies on isolated positive DGP showed low PPV for the DGP test (15.5% by Hoerter et al. [26] and 2.5% by Gould et al. [10]). Gould et al. [10] suggested that anti-DGP IgG should not be used as a screening test to diagnose CD. In our study, however, we had eight patients with negative tTG and positive DGP and showed a PPV of 75%. This should be interpreted cautiously due to the small sample size in our study. There is selection bias in all studies because the cases with low pretest probability of CD were not included in these studies, and precise evaluation requires a prospective study including cases with high and low pretest probability of CD and subsequent cases to determine if they finally develop CD.

In conclusion, this study indicated that anti-DGP IgG is a reliable serological test for the diagnosis of CD in children. In our study, the diagnostic accuracy of IgG anti-DGP for CD was superior to that of IgA anti-tTG. High tTG and DGP titers in the serum are suggestive of severe duodenal atrophy. The combined use of IgA anti-tTG and IgG anti-DGP tests for the initial screening of CD can improve the sensitivity.

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