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Clinical Value of Immunoglobulin A Antitransglutaminase Assay in the Diagnosis of Celiac Disease

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ABSTRACT

OBJECTIVES. Our goal was to evaluate the possible correspondence between anti-tissue transglutaminase of immunoglobulin A class levels and stage of mucosal damage in patients affected by celiac disease. In addition, we assessed clinical use of antitissue transglutaminase values to predict biopsy results.

METHODS. One thousand eight hundred eighty-six consecutive patients with symptoms suggestive of celiac disease and 305 healthy controls underwent determination of serum levels of immunoglobulin A and antitissue transglutaminase. An intestinal biopsy was performed in subjects with antitissue transglutaminase levels ≥ 4 IU/mL and in subjects with negative antitissue transglutaminase levels but with clinical suspicion of celiac disease. Histologic grading of celiac disease was consistent with the Marsh classification.

RESULTS. One hundred eighty-six subjects with positive antitissue transglutaminase levels and 91 patients with negative antitissue transglutaminase levels were submitted to biopsy. In all healthy subjects, antitissue transglutaminase results were negative. Histologic evaluations in patients with positive antitissue transglutaminase levels gave the following results: type 0 in 25 patients, type 1 in 3 patients, type 2 in 4 patients, type 3a in 22 patients, type 3b in 74 patients, and type 3c in 58 patients. None of the patients with negative antitissue transglutaminase levels showed histologic findings suggestive of celiac disease. The mean antitissue transglutaminase values in patients without mucosal atrophy were significantly lower than in patients with mucosal atrophy. Antitissue transglutaminase values ≥ 20 IU/mL were found in only 1 patient without mucosal atrophy.

CONCLUSIONS. Our study found a strong correspondence between antitissue transglutaminase levels and stage of mucosal injury; antitissue transglutaminase values >20 IU/mL seemed to be strongly predictive of mucosal atrophy.

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Key Words

celiac disease, antitissue transglutaminase, Marsh classification

Abbreviations

CD—celiac disease

Ig—immunoglobulin

EMA—antiendomysial antibodies of immunoglobulin A class

tTG—antitissue transglutaminase of immunoglobulin A class

PPV—positive predictive value

NPV—negative predictive value

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CELIAC DISEASE (CD) includes a continuous spectrum of histologic features, from high density of intra-epithelial lymphocytes to flat mucosa.¹⁻³ Antiendomysial antibodies of immunoglobulin (Ig)A class (EMA) in children showed a sensitivity from 88% to 100% and a specificity from 80% to 100%.^{2,4-11} Nevertheless, this test is assessed by an indirect immunofluorescent method and is expensive and operator-dependent.⁵

Antitissue transglutaminase of IgA class (tTG), the main autoantigen recognized by EMA,^{5,12,13} is now used to screen patients with CD.⁵ In patients presenting with IgA deficiency, tTG of IgG class determination is performed.^{5,14} tTG sensitivity and specificity using human recombinant protein range from 96% to 100% and from 84% to 100%, respectively, and both seem to be higher than tTG assay using guinea pig protein.⁵ tTG levels assessed by guinea pig assay in adult patients¹⁵ also seem to correlate with the degree of histologic damage.

In pediatric patients, it is not yet known whether a specific pattern of screening test could be consistent with the degree of mucosal damage. However, it was shown that mild values of tTG are associated with mild enteropathy in patients with CD.¹⁵ Our goal was to evaluate a

possible correspondence between tTG levels and stage of mucosal damage in patients with CD and to assess the clinical use of tTG levels to predict the results of intestinal biopsy.

MATERIALS AND METHODS

From January to December 2003, 1886 consecutive patients (905 boys and 981 girls ranging from 8 months to 17 years of age) were referred to our unit for the following clinical conditions: failure to thrive, iron deficiency, chronic abdominal pain, chronic diarrhea, vomiting, bloating, anorexia, asthenia, and positive familiar history for CD. All patients and controls underwent serum-level IgA and tTG determination. The controls were subjects who were submitted to minor surgical plastic procedures and who did not have gastrointestinal symptoms in their clinical history. The protocol used for the diagnosis is shown in Fig 1.

Total serum IgA was determined in patients using the Boeringher Mannheim Hitachi 912 analyzer, with Tinaquant reagents (Roche Diagnostics GmbH, Mannheim, Germany). tTG was measured with an enzyme-linked immunosorbent assay kit (Eu-tTG; Eurospital, Trieste,

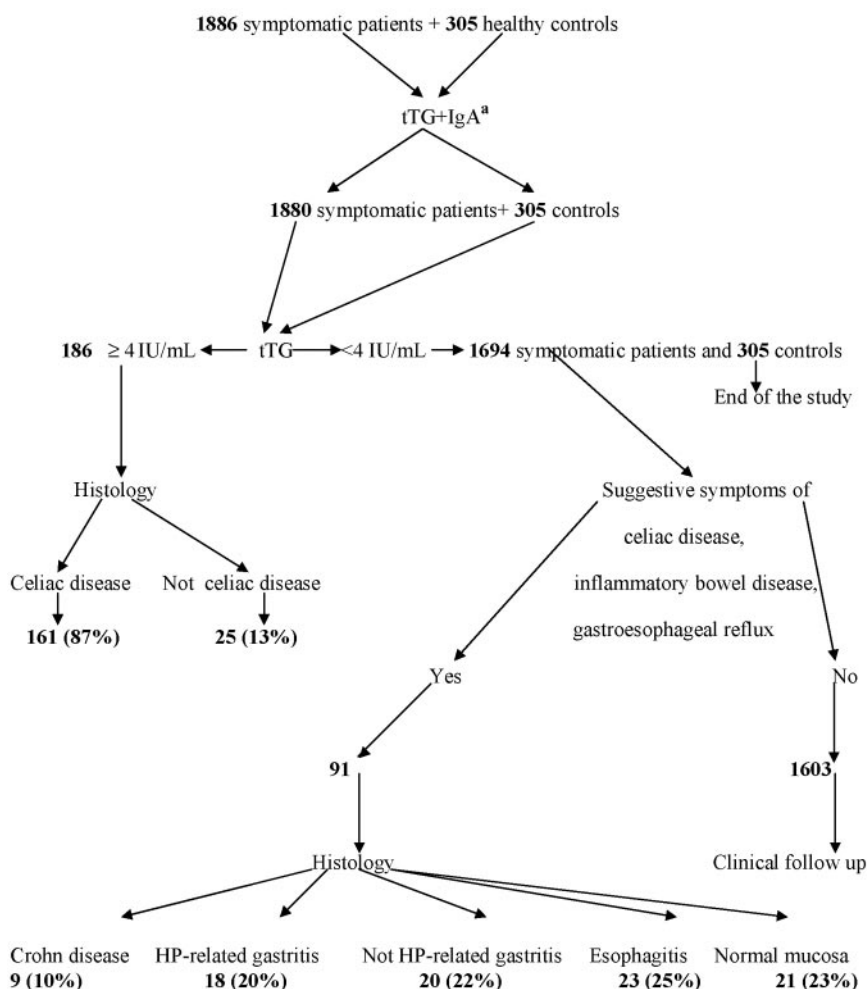


FIGURE 1
Diagnostic protocol and results of histologic evaluations. HP indicates *Helicobacter pylori*.^a Six symptomatic subjects were affected by IgA deficiency and were excluded from our study.

Italy). Antibody activity in the sera was calculated in international units per milliliter using a standard curve, and values ≥ 4 were considered to be borderline, whereas values >7 IU/mL were considered to be positive, according to the manufacturer's instructions. EMA was assessed using an indirect immunofluorescent method on a section of the lower one third of the monkey's esophagus (Applied Biosystems, Madrid, Spain). The antigen-antibody complexes were detected by means of fluorescein-labeled anti-human IgA and IgG on different slides and visualized with a fluorescent microscope. To determine HLA antigen type, Eu-DQ (Eurospital) was used as indicated in the manufacturer's instructions. An intestinal biopsy was performed in all subjects with tTG ≥ 4 IU/mL and in all tTG-negative subjects but with clinical suspicion of CD, gastroesophageal reflux, and inflammatory bowel disease.

The histologic grading of CD was expressed according to Marsh classification.¹⁻³ EMA and HLA antigen type (DQ2 and DQ8) determinations were performed in patients with disease classified as type 0, 1, and 2. Small intestinal biopsies were obtained during an endoscopic procedure. Three specimens from the distal segments of the duodenum were taken from each patient. Informed consent was obtained from the patients' families; the ethical committee of our hospital approved this study.

Our results were expressed as mean \pm SD; statistical analysis was made by *t* test and χ^2 test. *P* values $<.05$ were considered significant. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for tTG values, with intestinal biopsy as the gold standard.

RESULTS

During the study period, 6 subjects affected by IgA deficiency were excluded from our study; 186 subjects showing positive and borderline tTG values (66 boys and 120 girls; mean age: 7.1 ± 5.6 years; age range: 1-17 years) were submitted to intestinal biopsy.

Ninety-one tTG-negative patients (41 boys and 50 girls; mean age: 8.6 ± 6.6 years; age range: 1.8-17 years) underwent intestinal biopsy because of clinical suspicion

of CD, gastroesophageal reflux disease, and inflammatory bowel disease. The main clinical symptoms showed by patients submitted to intestinal biopsy were chronic diarrhea (35%), failure to thrive (27%), bloating (27%), chronic abdominal pain (22%), iron deficiency (18%), anorexia (15%), vomiting (12%), and asthenia (10%). Among tTG-positive patients, 8 patients (4%) had positive familiar history for CD. Clinical symptoms did not show significant differences between tTG-positive and tTG-negative groups. All control subjects showed negative tTG. The results of histologic evaluations in patients with positive, borderline, and negative tTG are shown in Fig 1. Table 1 shows diagnostic algorithm and treatment in patients with positive and borderline tTG. The results of EMA and DQ2/DQ8 detection are also shown.

Figure 2 shows mean values \pm SD of tTG within each histologic stage, in which tTG values were as follows: type 0, 11.6 ± 3.9 IU/mL (range: 4.8-19.7); type 1, 14 ± 6.1 IU/mL (range: 7.1-18.5 IU/mL); type 2, 15.5 ± 3.8 IU/mL (range: 12.3-20.8 IU/mL); type 3a, 18.7 ± 6.2 IU/mL (range: 6.4-28.4 IU/mL); type 3b, 20.1 ± 5.9 IU/mL (range: 4-30.3 IU/mL); and type 3c: 20.1 ± 5.4 IU/mL (range: 5.2-28 IU/mL). tTG mean values in type 3a, 3b, and 3c patients were significantly lower compared with tTG mean values in patients with type 0, 1, and 2 disease ($P < .0001$), as well as in patients with type 1 and 2 disease only ($P = .02$).

In our study, tTG values >20 IU/mL were observed in 1 patient with type 2 disease (25%), 12 patients with type 3a disease (55%), 42 patients with type 3b disease (57%), and 37 patients with type 3c disease (64%). tTG showed a sensitivity of 100% and specificity of 79% for cutoff values ≥ 4 IU/mL but a sensitivity of 99% and specificity of 85% for cutoff values ≥ 7 IU/mL. tTG values ≥ 20 IU/mL showed a PPV of 100% for mucosal atrophy. Table 2 shows sensitivity, specificity, PPV, and NPV for different tTG cutoff values.

DISCUSSION

In our patients with suspected CD, human recombinant tTG was used for the initial screening.⁵ Sensitivity was similar to values reported in other studies, although

TABLE 1 Diagnostic Algorithm and Treatment in 186 Patients With Positive tTG Assay Results Submitted to Intestinal Biopsy

Type ^a	No. of Patients (%)	No. of Patients With EMA Patients	No. of Positive DQ2/DQ8 Patients	Outcome
0	25 (13)	25	21	Normal diet ^b
1	3 (2)	3	3	Gluten-free diet
2	4 (3)	4	4	Gluten-free diet
3a	22 (12)	Not performed	Not performed	Gluten-free diet
3b	74 (39)	Not performed	Not performed	Gluten-free diet
3c	58 (31)	Not performed	Not performed	Gluten-free diet

^a According to Marsh classification.

^b These patients are being submitted to tTG and EMA detection every 6 months and will undergo additional biopsy if tTG and EMA are still positive 2 years after the first histological evaluation.

FIGURE 2
Mean values and SD of tTG values (IU/mL) in comparison with Marsh classification.

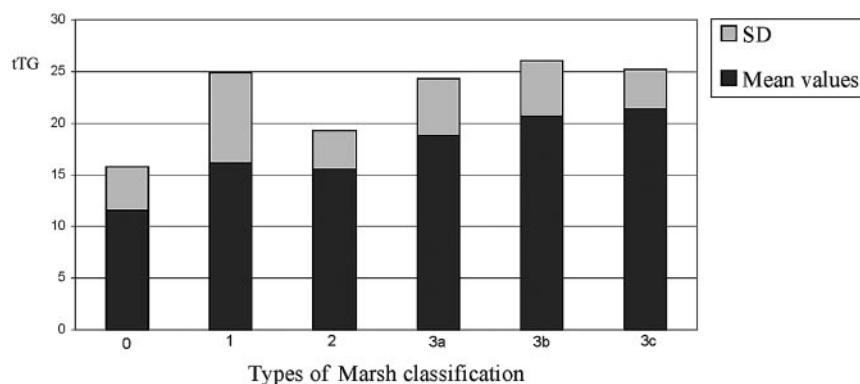


TABLE 2 Sensitivity, Specificity, PPV, and NPV of Different tTG Cutoff Levels

tTG Cutoff, IU/mL	Sensitivity, %	Specificity, %	PPV, %	NPV, %
≥4	100	79	88	100
≥7	99	85	90	99
≥20	50	100	100	49

specificity was lower.^{4,16–18} Therefore, our experience indicated a high prevalence of false-positive values of tTG compared with other studies. Patients with symptoms suggestive of CD and with tTG values ≥ 4 IU/mL, generally considered to be borderline, were submitted to intestinal biopsy. This choice probably reduced the specificity of our tTG assay. DQ2 or DQ8 were found positive in many patients with type 0 disease, who showed positive EMA in all cases. Therefore, according to other studies,^{7,8,10,19} patients with type 0 disease can be considered in our experience as “potential CD.” It is known that patients without histologic damage but with immunologic abnormalities need to undergo repeated biopsies under normal gluten consumption to show a progression toward the typical mucosal changes.^{7,8,10,19} Many studies show that EMA is the best screening test for predicting the progression toward villous atrophy,⁵ but the possible role of tTG as a predictor of developed mucosal atrophy is not yet known. In our experience during the follow-up, 3 of 25 patients with type 0 disease showed a reduction of tTG level but maintained the EMA positiveness. Our patients with type 0 disease will require careful follow-up. These patients will undergo multiple biopsies after normal gluten consumption if they are still found tTG-positive and EMA-positive, because the first normal biopsy could represent a missed biopsy resulting from the patchy nature of the lesion in the early stages.^{2,6,9,20,21} Patients with type 1 and 2 disease, all tTG-positive and EMA-positive, and all HLA antigens compatible with CD were also submitted to a gluten-free diet with clinical recovery. All of our patients with mucosal atrophy started a gluten-free diet, with complete resolution of the symptoms.

To our knowledge, this is the largest study conducted in pediatric patients that considers tTG levels according

to Marsh classification. However, a correlation between tTG levels and mucosal injury in patients with CD was already suggested by other studies.^{22–24} Fabiani et al²³ showed significantly lower levels of tTG in patients with mild enteropathy than in those with more severe enteropathy. Hansson et al,²⁴ in a study conducted in 57 children with biopsy-verified CD, showed serum tTG levels to be significantly increased in patients with mucosal atrophy compared with patients with partial or subtotal villous atrophy. In a previous series referring to adult patients,²² tTG levels were also found to be strongly correlated with the histologic degree of mucosal damage, as in our pediatric patients with pediatric CD. In the adult series, however, tTG was screened by enzyme-linked immunosorbent assay using guinea pig liver substrate, whereas in our study human tTG was used. The similar outcome of the tTG assay, although assessed by different substrates and by different methods,^{22–24} strengthens our assumption that tTG values are important in predicting mucosal atrophy in symptomatic patients. The limit of our work is represented by the small number of patients with type 1 and 2 disease with CD, in comparison with the study on adult patients.²²

In our experience, human tTG assay, used as a screening test of suspected patients with CD according to the recommendations of North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition,⁵ showed high sensitivity. tTG values ≥ 20 IU/mL were strongly predictive of mucosal atrophy, whereas tTG values < 20 IU/mL seemed to require both histologic confirmation (because of the possibility of false-positive) and EMA and HLA antigen assessment. According to our results in a recent study of a selected pediatric population, patients with very high tTG levels were false-positive for CD and, therefore, able to replace small bowel

biopsy.²⁵ According to Barker et al,²⁵ we suggest that a multicenter study be performed, as conducted by Collin et al,²⁶ to achieve a definite conclusion about the performance of tTG in predicting mucosal damage.

CONCLUSIONS

Our study suggests that there is a strong correspondence between tTG levels and the stage of mucosal injury in patients with symptoms suggestive of CD; tTG cutoff values >20 IU/mL also seem suitable for predicting the mucosal atrophy.

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