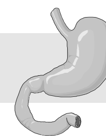

Comparison of Assays for Anti-endomysial and Anti-transglutaminase Antibodies for Diagnosis of Pediatric Celiac Disease

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Key words: transglutaminase, tissue transglutaminase, celiac disease, anti-endomysial antibody

For Editorial see page 82

Abstract

Background: Anti-endomysial antibodies are sensitive and specific markers for celiac disease. This antibody has recently been identified as an antibody to tissue transglutaminase, an enzyme that cross-links and stabilizes extracellular matrix proteins.

Objectives: To evaluate the clinical usefulness of an enzyme-linked immunoassay for anti-transglutaminase antibodies, and to compare the results with those of AEA, the current gold standard serological test for celiac disease.

Methods: Serum samples were collected from 33 patients with biopsy-proven celiac disease and AEA tests were performed. Control samples for anti-transglutaminase were obtained from 155 patients. An ELISA test for immunoglobulin A anti-transglutaminase utilizing guinea pig liver transglutaminase was developed and performed on all sera. Cutoff values for the test were performed using logistic regression and receiver operating curves analysis.

Results: An optical density cutoff value of 0.34 was established for the assay. The mean value was 0.18±0.19 optical density for controls, and 1.65±1.14 for patients with celiac disease ($P<0.001$). Sensitivity and specificity of the assay were both 90%, while AEA had a sensitivity and specificity of 100% and 94%, respectively.

Conclusions: A tissue transglutaminase-based ELISA test is both sensitive and specific for detection of celiac disease.

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AEA = anti-endomysial antibody

Celiac disease is a disorder characterized by small intestinal inflammation, damage to villi, and ensuing malabsorption. It can present with intestinal or extra-intestinal manifestations [1]. The gold standard for diagnosis is small intestinal biopsy demonstrating inflammation and villous atrophy. Serological indicators, which are markers of active disease, are used for initial diagnosis prior to biopsy, and for follow-up of compliance with a gluten-free diet. Tests currently in use rely on detection of circulating antibodies, and include anti-endomysial antibody and anti-gliadin antibody. The sensitivity and specificity of AEA approach 95%–100% and are considered to be significantly better than those of AGA with sensitivity and specificity ranging between 65% and 97% [2–5]. However, AEA is an indirect immunofluorescence test requiring monkey esophagus or human umbilical cord.

Dietrich et al. [6] recently discovered that AEA is directed against tissue transglutaminase, an enzyme that cross-links glutamine and lysine residues in extracellular matrix proteins. To test this hypothesis, they developed an ELISA for detection of IgA anti-tTG antibodies and tested it on 12 patients with celiac disease and a positive AEA. These findings are of major importance in understanding the pathogenesis of the disease and the presence of AEA. Since our aim was to evaluate the clinical utility of this test, we performed it in a larger group of patients and in controls to establish the sensitivity and specificity of the assay.

Methods

Samples of serum from two pediatric gastroenterology clinics in the Tel Aviv area in Israel (Wolfson Medical

AGA = anti-gliadin antibodies
tTG = tissue transglutaminase

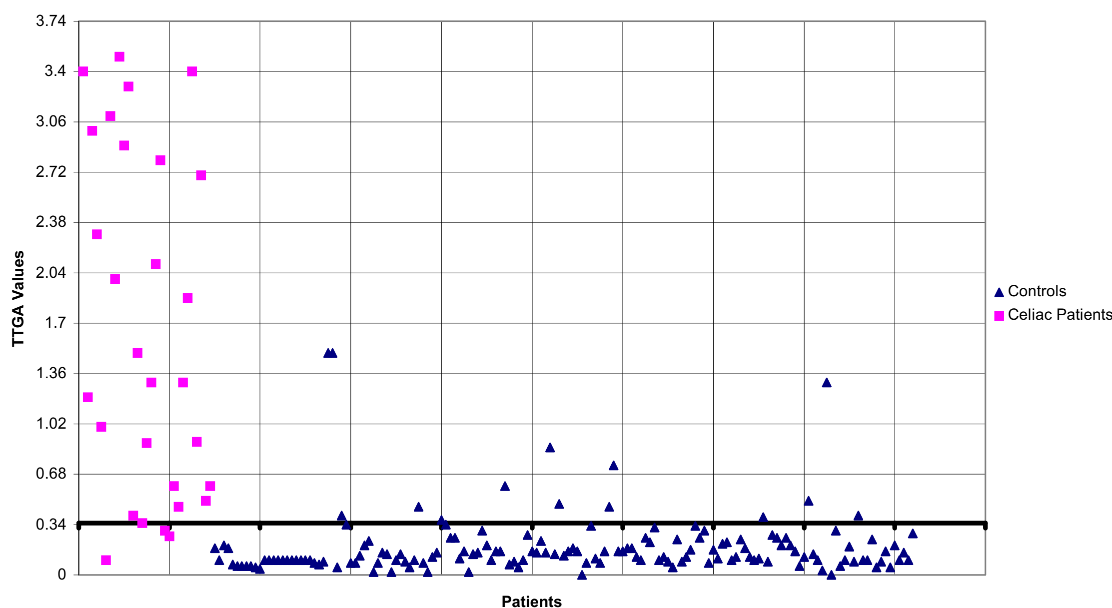

Figure 1. Distribution of TTGA values in celiac disease and controls.

Center in Holon, and Dana Children's Medical Center in Tel Aviv) that were sent for AEA testing were frozen at -20°C. Celiac disease was diagnosed by positive AEA, by small intestinal biopsy showing typical findings of villous atrophy and inflammation, and by clinical and serological response to a gluten-free diet. Remnants of blood sent for blood chemistry from a pediatric emergency room were collected, and serum was frozen at -20°C. Samples collected from patients aged 1–18, without known celiac disease, presenting with an acute febrile illness or trauma, were used as controls. AEA testing was performed in control sera if tTGA was high. Patient recall was performed in controls if both tTGA and AEA were elevated. AEA results were scored as positive, negative or borderline (detectable and weak at a titer of 1/5, negative <1/5). AGA was considered positive if both IgA and IgG were elevated. The study was approved by our hospital's ethics committee.

AEA was performed using the AEA Test (IMCO Diagnostics, Buffalo, NY, USA). ELISA for the detection of IgA antibodies to tTG was performed according to the method of Dietrich et al. [6], and modified. Briefly, 96-well microtiter plates (Nunc, Denmark) were incubated with tTG (Sigma, USA) using 1 μg tTG/100 μl phosphate-buffered saline/well, for 2 hours at 37°C. Unsaturated sites were blocked using PBS containing 1% bovine serum albumin, and incubated overnight at 4°C. Sera were diluted (1/100) with PBS 0.1% Tween-20 (ICN Biomedicals Inc. Ohio, USA) and incubated for an hour at room temperature. Following three washes with PBS 0.1% Tween-20/0.1% azid, wells were reacted with 100 μl of peroxidase conjugated anti-human IgA (Jackson Immunoresearch Laboratories, PA, USA) diluted in PBS 0.1% Tween-20/0.1% azid for an hour at room temperature. Unbound conjugate was removed by three washes with PBS 0.1% Tween-20/0.1% azid. Color reaction was developed by the addition of substrate buffer 100 μl/well containing 0.4 mg/ml 0-phenylenediamine-hydrochloride in 0.05 M phosphate-citrate buffer pH5 and 40 μl fresh 30% hydrogen peroxide/100 μl of substrate buffer solution added prior to use. Optical density at 490 nm was measured by ELISA Reader (Molecular Devices, Israel).

Statistical analysis

ANOVA was performed to find significant differences between groups. Square root transformation of TG values was performed prior to ANOVA to improve distribution of values. *P* values <0.05 were considered significant. Logistic regression and receiver operating curves (ROC Analysis) were performed to determine the cutoff point for TG providing the optimal levels of sensitivity and specificity. All calculations were performed using BMDP statistical software (BMDP Statistical Software, 1990, University of California Press, USA).

Results

Sera from 33 celiac patients and 155 controls were available for tTGA analysis. Three celiac patients compliant with a gluten-free diet had negative AEA and low tTGA levels. These patients were excluded from further analysis.

tTGA = tTG antibodies
PBS = phosphate-buffered saline

TG = transglutaminase

Table 1. Sensitivity, specificity and predictive values for serologic tests in celiac disease

	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
tTGA	90	90	64	91
AEA	100	94	98	100
AGA	100	75		

Using ROC analysis, the cutoff level for a positive test chosen was an OD reading of >0.34, in order to achieve the maximum specificity with a minimal sensitivity and specificity exceeding 85%.The mean value for controls (mean±standard deviation) was 0.19±0.22 OD vs 1.67±1.15 OD for patients with active disease ($P<0.001$). Data from patients and controls are presented in Figure 1.

Anti-endomysial results were available from 80 patients. Results for AGA as well as tTGA and AEA were available from 44 control patients.

All 30 patients with celiac disease had detectable AEA. Three patients (10%) with AEA-positive celiac disease had normal or borderline tTGA results. In two of three, AEA was also borderline. Positive tTGA in excess of 0.34 was found in 15 of 155 (10%) controls. Three of these control patients (3 of 15 positive tTGA) had positive AEA. Two patients with positive AEA had the test repeated twice and small intestinal biopsies were interpreted as completely normal. Another patient with a borderline AEA refused biopsy. These patients were considered controls for statistical evaluation. Sensitivity and specificity were calculated for the three assays and are shown in Table 1.

Discussion

Current diagnostic criteria for the diagnosis of celiac disease were outlined in 1990 by ESPGAN (European Society for Pediatric Gastroenterology and Nutrition) and include the requirement of a small bowel biopsy demonstrating the characteristic histology of celiac disease and clinical resolution on a gluten-free diet [7]. Serological markers serve as useful screening tools to select patients for biopsy and to follow compliance with a gluten-free diet. Cataldo et al. [8] demonstrated that AEA is the most useful marker for both these indications. The problems associated with AEA testing are the use of monkey esophagus and the relative expense of the kits. An alternative test using human umbilical cord as a substrate for AEA has sensitivity and specificity equivalent to conventional tests using monkey esophagus [4].

With the identification of tTG as the major autoantigen of celiac disease, along with a method for measuring tTGA [6], a test that directly detects antibodies considered specific for the disease could become clinically useful. With that in mind we evaluated the clinical utility of the assay.

tTGA in fact can differentiate between patients with celiac disease and controls. Mean tTGA values in patients were significantly higher (1.65±1.14 OD vs. 0.18±0.19 OD, $P<0.001$) for patients with active disease. Patients compliant with a gluten-free diet had low tTGA, as well as negative AEA. The specificity was high (90% at a cutoff value of 0.34), though lower than that of AEA (94%). For patients with a value of 1.0 or greater, specificity was 94%, equal to that of AEA. Even though tTGA might have a slightly lower specificity than AEA, an additional higher numerical cutoff point could be used to achieve higher specificity in selected patients.

tTGA, however, is less sensitive than AEA. tTGA was negative in 10% (3 of 30) of active celiac patients, while AEA was positive (though weakly so in 2 of 3) in these patients. Guinea pig liver tTG has 80% homology for human tTG [6], and this conceivably may play a role in decreased binding of tTGA to tTG in the assay. This may be more significant with low level titers, such as demonstrated in our study. An AEA titer of <1/5 is considered negative by many laboratories, and these patients would be read as negative by AEA as well. IgA deficiency, reported in approximately 2% of celiac disease patients [9,10], and cited as a reason for decreased sensitivity of AEA, is unlikely to have played a role since all patients with celiac disease included in the study were IgA AEA positive. We did not assess quantitative IgA levels, and it is possible that IgA levels were lower in these three patients. Use of a cutoff point of 0.1 such as used by Troncone et al. [11] would have detected these patients, but in our assay would have lowered specificity below the targeted 85%.

In comparison to AGA, albeit with lower numbers examined, tTGA had superior specificity but lower sensitivity. The high sensitivity of AGA in our study may be biased, as AGA was included for analysis only in patients with both AGA and AEA. Positive AGA may have led to subsequent performance of AEA for confirmation. The fact that AEA was performed in only 80 patients and in tTGA-positive controls may also have added to a bias in the data.

It is interesting to note that the two patients from the control group who had repeatedly positive AEA had elevated tTGA as well. Small bowel biopsies were normal in both patients, and there was no apparent increase in intra-epithelial lymphocytes. This could reflect either non-specific elevation of the antibody or latent celiac disease. Troncone [12], reviewing latent celiac disease in Italy, noted cases with apparent normal biopsy, without an increase in intra-epithelial antibodies [12]. In a study published by Cataldo et al. [8], five patients in a control group with positive AEA and normal biopsies had second biopsies within 18 months and were found to have flat mucosa, indicative of celiac disease. We did not apply other markers for latent celiac disease.

Our results are very similar to those recently published by Troncone and colleagues [11]. They found a concordance rate of 95% between tTGA and AEA, with sensitivity and positive predictive values >90%, but slightly less

ROC = receiver operating curves

than AEA. Our assay provided a lower positive predictive value and similar negative predictive value to those published in Troncone's study.

tTGA is a useful test for the detection of celiac disease and may replace AEA testing in the future. An ELISA test with high sensitivity and specificity could be superior to assays in use today, and may overcome obstacles such as ethical issues related to the use of monkeys, subjectivity in interpretation, and higher costs related to these tests. Though sensitivity and specificity of an IGA ELISA test for tTGA in our study were high, they were still slightly inferior to those of AEA.

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