

Tissue Transglutaminase Enzyme-Linked Immunosorbent Assay as a Screening Test for Celiac Disease in Pediatric Patients

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ABSTRACT. *Objective.* An immunoglobulin A (IgA) anti-tissue transglutaminase antibody assay (anti-tTG) was compared with the conventional IgA anti-endomysium antibody assay (EMA) to assess its reliability as a screening test for celiac disease (CD) in a pediatric population.

Methods. Seventy-five IgA-sufficient and 2 IgA-deficient children who were scheduled for small intestinal biopsy for the evaluation of history or symptoms suggesting a diagnosis of CD were prospectively evaluated and enrolled in this study (gastrointestinal [GI] patients). In addition, 16 children with type I diabetes mellitus (DM) who had a positive EMA and a small bowel biopsy were included as a separate cohort. IgA anti-tTG was measured by enzyme-linked immunosorbent assay (ELISA), and IgA-EMA titers were determined by indirect immunofluorescence on cryopreserved sections of monkey esophagus.

Results. Nine of the 75 IgA-sufficient GI patients had a small bowel biopsy consistent with the diagnosis of CD. Eight of 9 IgA-sufficient patients with a positive small bowel biopsy had positive anti-tTG and EMA tests. Four IgA-sufficient patients had a false-positive anti-tTG ELISA and 2 had a false-positive IgA-EMA assay. In the IgA-sufficient patients, the sensitivity was 89% and the negative predictive value was 98% for either assay. The specificities of the IgA anti-tTG and the IgA-EMA tests were 94% and 97%, respectively (not significant). The positive predictive value of the IgA anti-tTG was 67%, compared with 80% for the IgA-EMA (not significant). In the 2 IgA-deficient children, one of whom had biopsy-proved CD, both tests were negative. In the 16 DM children 12 true- and 4 false-positive IgA anti-tTG and IgA-EMA results were identified. Three of 12 complained of GI symptoms. In follow-up, thus far, none of the DM patients with a false-positive anti-tTG have developed CD.

Conclusions. The IgA anti-tTG antibody assay is equivalent to the IgA-EMA assay as a screening test for CD in IgA-sufficient pediatric patients. Intestinal biopsy remains the gold standard for the diagnosis of CD. *Pediatrics* 2001;107(1). URL: <http://www.pediatrics.org/cgi/content/full/107/1/e8>; celiac disease, tissue transglutaminase, endomysium antibody, diagnosis, screening tests.

ABBREVIATIONS. CD, celiac disease; DM, diabetes mellitus; IgA, immunoglobulin A; EMA, endomysium antibody; IIF, indirect immunofluorescence; tTG, tissue transglutaminase; ELISA, en-

zyme-linked immunosorbent assay; anti-tTG, antibody to tissue transglutaminase; GI, gastrointestinal; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Celiac disease (CD) is a common cause of malabsorption in children. It is characterized by mucosal atrophy in the small intestine that is reversible with adherence to a strict gluten-free diet. Studies have shown that the long-term risk of growth failure, osteoporosis, and intestinal malignancy, such as lymphoma, may be reduced with dietary treatment.^{1,2} Early diagnosis and management are, therefore, important in reducing both morbidity and mortality.

Referrals to pediatric gastroenterologists to evaluate patients with suspected CD present a diagnostic challenge. The complexity of the clinical presentation is appreciated when it is understood that CD may present in the context of first-degree relatives of a patient with CD, type 1 diabetes mellitus (DM), autoimmune thyroiditis, short stature, delayed puberty, trisomy 21, unexplained iron/folate deficiency, alopecia areata, adrenal insufficiency, seizures with cerebral calcification, peripheral neuropathy, autoimmune connective tissue disease, dermatitis herpetiformis, sclerosing cholangitis, dental enamel hypoplasia, immunoglobulin A (IgA) nephropathy, and microscopic colitis.^{1,3}

Although definitive diagnosis relies on intestinal biopsy, IgA anti-endomysial autoantibody (EMA) assays are a reliable serologic screening test. A number of studies have generally found IgA-EMA detection to be highly sensitive (85%–100%) and specific (90%–100%), although sensitivities as low as 60%⁴ and 55%⁵ have recently been described. Nevertheless, determination of a positive or negative IgA-EMA can be difficult and is fraught with false-negative results because it relies on indirect immunofluorescence (IIF) staining of tissues containing the intermyofibrillar substance of smooth muscle. IIF assays are notably labor-intensive, semi-quantitative, difficult to interpret when other autoantibodies are present, and rely on subjective interpretation, introducing the possibility of interobserver variability.⁶ Tissue substrates commonly used for conventional EMA IIF assays include cryopreserved sections of monkey esophagus or human umbilical cord.⁷ The use of monkey esophagus is increasingly problematic because of short supply and ethical concerns about the procurement of these tissues.

Recent identification of tissue transglutaminase (tTG) as the predominant autoantigen targeted by

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EMA has led to the development of alternative assays that include a quantitative enzyme-linked immunosorbent assay (ELISA) that measures IgA antibodies to tTG (anti-tTG) and theoretically overcomes some of the problems encountered with EMA. A number of studies of patients with known CD have compared the 2 assays in an attempt to determine whether IgA anti-tTG ELISA can replace the EMA IIF assay.^{5,8,9} These studies have generally shown that IgA anti-tTG ELISA has sensitivity ranging from 85% to 100% and specificity from 90% to 100%. The 2 screening tests have shown good quantitative correlation ($r = 0.86$) as well as strong concordance (95%). However, most of these studies did not focus on children and were a retrospective analysis of patients with an established diagnosis of CD.

We prospectively evaluated the sensitivity and specificity of an IgA anti-tTG ELISA as a screening test in the diagnosis of CD in children undergoing intestinal biopsy at a pediatric gastrointestinal (GI) clinic. Results of this assay were compared with IgA-EMA test results and the results of a small intestinal biopsy.

METHODS

Participants

The Conjoint Medical Research Ethics Board of the University of Calgary approved this study. Sera were obtained from 93 children who were scheduled to undergo small intestinal biopsy at the Alberta Children's Hospital GI clinic from October 1995 to May 1999. The study population consisted of 77 children (GI group, 2 months to 16 years of age) being evaluated prospectively for abdominal pain ($n = 19$), diarrhea (19), failure to thrive/short stature (16), family history of CD (12), Crohn's disease (5), vomiting (2), abdominal distension (1), ulcerative colitis (1), autoimmune thyroiditis/short stature (1), and trisomy-21 (1). Sera in this GI group were obtained prospectively.

In addition, sera from 16 children 3 to 18 years of age with type 1 DM with a known positive IgA-EMA and either positive ($n = 12$) or negative (4) intestinal biopsies for CD were tested retrospectively for IgA anti-tTG. The 16 patients have been previously reported in a study that noted a high false-positive rate with IgA-EMA assays in patients with type 1 DM.¹⁰

Autoantibody Detection

Sera obtained from all participants were screened for IgA anti-tTG and EMA. The IgA-EMA test system (SCIMEDX Corporation, Denville, NJ) was used as previously described.¹⁰ This IIF method used cryopreserved sections of monkey esophagus as substrate, and sera were serially diluted from 1:10 to 1:640. Two experienced technicians who were blinded to the patient's history or to the results of the small bowel biopsy examined the slides. As established by the manufacturer, typical staining of the endomysium at titers of 1:10 or greater was considered a positive test. IgA anti-tTG antibody detection based on antigen purified from guinea pig liver used a commercial anti-tTG ELISA kit (QuantaLite, INOVA, San Diego, CA). Standardized optical density values of >20 were considered positive for IgA anti-tTG antibodies as established by the manufacturer. Total serum IgA was measured using rate nephelometry.

Intestinal Biopsy and Histopathological Analysis

All 93 patients underwent small intestinal biopsy by an experienced pediatric gastroenterologist. Two methods were used to obtain small intestinal mucosal biopsies: a Carey capsule, as previously described,¹⁰ or 4 to 6 duodenal biopsies obtained at the time of endoscopy. An experienced pathologist who was blinded to the patient's history and antibody assay results assessed the mucosal biopsy sections for pathologic features of CD that included villus atrophy, crypt hyperplasia, increased intraepithelial lymphocytes, and chronic inflammation in the lamina propria. A

diagnosis of CD was made when there was an increased number of intraepithelial lymphocytes with associated subtotal or total villus atrophy.¹¹

Analyses

A 2-sample test of proportions (McNemar's test, Stata for Windows, Stata, College Station, TX) was used to compare proportions between screening assays. Spearman's correlation coefficient was determined as well as concordance rate (number of matching IgA anti-tTG and IgA-EMA assay outcomes/total number of participants) to give an indication of the degree of agreement between the 2 tests. Sensitivity (number of patients with positive test and biopsy/total number of patients with biopsy-proven CD), specificity (number of patients with negative test and biopsy/total number of patients with a biopsy negative for CD), positive predictive value (PPV; number of patients with positive biopsy and test/total number of patients with positive test), and negative predictive value (NPV; number of patients with negative biopsy and test/total number of patients with negative test) were calculated from the GI group data for IgA anti-tTG and IgA-EMA assays.

RESULTS

Two of the 77 GI participants were found to be IgA-deficient, one of whom was diagnosed with CD based on the biopsy. Both tested negative for IgA-EMA and IgA anti-tTG. Of the remaining 75 IgA-sufficient patients, 9 (12%) were positive for CD on biopsy. Both anti-tTG and EMA assays identified 8 true-positives and 1 false-negative in IgA-sufficient patients. The IgA-sufficient false-negative patient had chronic diarrhea and signs of malabsorption. Anti-tTG ELISA identified 62 true-negatives and 4 false-positives, compared with 64 true-negatives and 2 false-positives in the EMA assays, in IgA-sufficient patients.

Sensitivity, specificity, PPV, and NPV of both tests in the IgA-sufficient GI group did not differ significantly (Table 1). IgA-deficient patients were excluded in this analysis. IgA anti-tTG and EMA assays had identical sensitivities of 89% (8/9; 95% confidence interval [CI]: 52–99.7) and similar specificities of 94% (62/66; 95% CI: 85–99) and 97% (64/66; 95% CI: 89–99.6), respectively. PPV for IgA anti-tTG ELISA was 67% (8/12; 95% CI: 35–90), which did not differ significantly from the PPV of the EMA assay of 80% (8/10; 95% CI: 44–97). NPV was similar for both tests at 98% (62/63; 95% CI: 91–99.9) for the anti-tTG assay and 98% (64/65; 95% CI: 92–99.9) for the EMA assay. The 2 tests showed high correlation ($r = 0.81$) and concordance (97%) in the GI group.

Of the 16 IgA-EMA-positive DM patients, 12 were found to have CD on biopsy. None of these patients was IgA-deficient. Both assays produced identical results with 12 true-positives and 4 false-positives. The 2 assays demonstrated high correlation ($r = 0.79$) and 100% concordance in this group.

A summary of the false-positive and false-negative results is shown in Table 2. In the GI group, both

TABLE 1. Specificity, Sensitivity, PPV, and NPV of IgA Anti-tTG and EMA Assays in GI Group

Assay	Specificity % (95% CI)	Sensitivity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Anti-tTG	94 (85–99)	89 (52–99.7)	67 (35–90)	98 (91–99.9)
EMA	97 (89–99.6)	89 (52–99.7)	80 (44–97)	98 (92–99.9)

TABLE 2. False-Positive and False-Negative Assay Results in GI and DM Groups

Patient Number	Group	IgA Anti-tTG Titres	IgA-EMA Dilution	Biopsy
1	GI	22	0	Normal
2	GI	33	0	Normal
3	GI	60	1:10	Normal
4*	GI	257	1:20	Increased IEL
5	GI	15	0	CD
6	GI	7.7	0	CD, IgA-deficient
7	DM	45	1:10	Normal
8	DM	57	1:10	Normal
9	DM	85	1:20	Normal
10	DM	304	1:80	Normal

IEL indicates intraepithelial lymphocytes.

* Later developed biopsy-confirmed CD with a 1:320 EMA titre.

tests produced 2 false-negative results (patients 5 and 6). Patient 6 was IgA-deficient. Four false-positives (patients 1–4) were also obtained, with 2 being positive in both assays (patients 3 and 4) and 2 being positive in the IgA anti-tTG assay only (patients 1 and 2). Patients 1 and 2 had very low IgA anti-tTG titers. The biopsy of patient 4 showed increased intraepithelial lymphocytes, and the patient developed CD with high IgA-EMA titers (1:320) at a later date.

In the DM group, both tests produced 4 false-positive results (25%). Three of the 4 false-positives (patients 7, 8, and 9) had low to moderate IgA-EMA and IgA anti-tTG titers; the fourth patient (patient 10) had high antibody levels in both assays. With all study participants, the IgA anti-tTG and IgA-EMA assays showed a high overall correlation ($r = 0.85$) and concordance (98%). None of the false-positives in this group have developed biopsy-proven CD.

DISCUSSION

The recent identification of tissue transglutaminase (tTG) as a key autoantigen in CD has facilitated the introduction of a potentially improved serologic screening tests for this disease. tTG is a calcium-dependent enzyme that cross-links specific collagen types and fibronectin during tissue injury.¹² The potential role of a quantitative IgA anti-tTG ELISA as a replacement for the more time consuming and subjective IgA-EMA indirect immunofluorescence assay has important implications in terms of cost reduction and international standardization of results.

Many retrospective studies have compared IgA anti-tTG ELISA with IgA-EMA-IIF in adult CD populations, and high specificities and sensitivities have been demonstrated.^{5,8,9} Our study of children prospectively compared an IgA anti-tTG ELISA with a conventional IgA-EMA-IIF assay in children undergoing small intestinal biopsy in a GI clinic. We observed high overall concordance between the 2 tests, with discordance noted in only 2 of 93 patients. The high correlation between the assays is consistent with previous studies.^{5,8,9}

In our pediatric GI group, the assays showed similar specificity, sensitivity, PPV, and NPV. The true-negative assay results in children with Crohn's disease, ulcerative colitis, diarrhea, and failure to thrive suggest that both IgA anti-tTG and EMA assays are

specific in the presence of other GI pathology. Of 4 false-positive results, 3 of the patients belonged to high-risk groups for CD (2 with close family history; 1 with trisomy-21). The patient with trisomy-21 had the highest IgA anti-tTG and IgA-EMA titers and biopsy demonstrated early intestinal pathology at the time of the study; this patient eventually developed clinically overt CD that substantiated by a subsequent biopsy. This case illustrates that the detection of IgA anti-tTG antibodies may be an economical screening test for subclinical disease. Of the 2 false-negatives, one was IgA-deficient. The other may be explained by the existence of autoantibodies specific for certain human, but not animal, antigens that may not be detected by the animal substrate-based assay.

The study also retrospectively evaluated a subset of diabetic children, a group that has a higher prevalence of CD.^{10,13,14} It has been suggested that the association between type I diabetes and CD is attributable to an immune dysfunction and altered dietary protein tolerance caused by nonspecific activation of the immune system.¹⁵ In the DM group, IgA anti-tTG and EMA assays produced matching results. The IgA anti-tTG ELISA was unable to reduce the number of false-positives, compared with the IgA-EMA assay, because a significant percentage (25%) of the DM population with a positive screening test yielded false-positive results with both assays.

The apparent false-positive assay results in both GI and DM groups may reflect either true false-positives or patients at risk of progressing to CD.^{13,16,17} Studies have shown that serologic markers used to screen for CD may be predictive of latent disease in high-risk patients, such as those with type I DM.^{16–18} Such studies report patients with positive serum IgA-EMA but normal biopsies who later developed biopsy-confirmed CD. Of interest, 1 of the 2 patients in our GI group that initially had a positive tTG and EMA, 2 years later had increased antibody titers and a positive small bowel biopsy. In addition to the problem of subclinical disease as evidenced by normal biopsies, there are several other explanations for false-positive test results. One is the presence of autoantibodies that bind to other autoantigens in tissue substrates or to trace contaminants in purified preparations of tTG. To avoid these problems, assays based on purified human recombinant tTG are being developed. The performance of these assays in a prospective clinical setting may be an improvement over current protocols.

CONCLUSION

The IgA anti-tTG ELISA used in our study is equivalent to a conventional IgA-EMA IIF assay as a screening test for CD in IgA-sufficient pediatric patients presenting with suspected GI disease. False-positive patients should be followed with serial antibody testing because they may represent a subpopulation of patients with latent CD that develop the disease at a later date. Although the tTG ELISA may offer advantages of speed and the capacity to analyze a large number of serum samples, the intestinal biopsy remains the gold standard for the diag-

nosis of CD in children with suspected malabsorption.

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