



Positive Tissue Transglutaminase Antibodies with Negative Endomysial Antibodies: Low Rate of Celiac Disease

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Abstract

Background: Screening for celiac disease is based on the sequential evaluation of serologic tests and intestinal biopsy; an optimal screening protocol is still under investigation. The screening policy of one of the main healthcare providers in Israel (Maccabi) consists of measuring total immunoglobulin A and tissue transglutaminase IgA antibodies and confirming positive results by endomysial antibodies. For IgA-deficient patients antigliadin IgG is measured.

Objectives: To evaluate the use of tTGA as a first-level screening test in patients suspected of having celiac disease

Methods: The results of tTGA and EMA tests over a 3 month period were obtained from the laboratory computer. Letters were sent to the referring physicians of patients with positive tests, requesting clinical information and small intestinal biopsy results. tTGA was performed using an anti-guinea pig tTG-IgA enzyme-linked immunosorbent assay kit.

Results: Overall, 2,505 tTGA tests were performed: 216 (8.6%) were tTGA-positive of which 162 (75%) were EMA-negative (group 1) and 54 (25%) EMA-positive (group 2). Clinical information was obtained for 91 patients in group 1 and 32 in group 2. Small intestinal biopsy was performed in 33 (36%) and 27 patients (84%) in groups 1 and 2, respectively. Celiac disease was diagnosed in 4 biopsies (12%) in group 1 and 23 (85%) in group 2 ($P < 0.0001$). The positive predictive value was 45% for tTGA and 85% for EMA.

Conclusions: Symptomatic patients with positive tTGA and negative EMA have a low rate of celiac disease compared to those who are tTGA-positive and EMA-positive. Confirmation with EMA is advised when tTGA is performed as a first-level screening for suspected celiac disease

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Celiac disease is a common permanent intolerance to gluten that occurs in genetically predisposed individuals and is triggered by immune-mediated mechanisms. The disease is increasingly recognized in Europe and the United States, with a prevalence of 1:250 individuals in the general population, or higher [1-4]. A recent

study of a population of blood donors in Israel found a prevalence of at least 1:157 [5]. The reason for the lack of diagnosis based on clinical symptoms is that most patients have silent disease or minimal non-specific symptoms [6].

The diagnosis of celiac disease has traditionally required a mucosal biopsy of the proximal small bowel. Serologic screening tests are currently being used to select candidates for intestinal biopsy. Immunoglobulin-A anti-endomysial antibodies are considered the best immunologic marker, with almost 100% sensitivity and specificity in untreated patients [7]; however, this test is observer-subjective, cumbersome, time-consuming and expensive for large-scale screening. When used as a screening test of asymptomatic patients EMA may underestimate the prevalence of the disease [8,9]. Tissue transglutaminase was recently identified as the autoantigen recognized by EMA [10], and a number of enzyme-linked immunosorbent assay commercial kits were developed to determine the serum anti-tTG antibodies. The antigen in the kit may be guinea pig tTG or recombinant human tTG. Anti-tTG antibodies were found to have 90-100% sensitivity and 94-100% specificity for celiac disease, and a good correlation with EMA [11-15]. The potential use of tTG antibodies to screen for the disease has been investigated, but it is still unknown whether it can replace EMA [16,17]. The use of one serologic marker was found to be insufficient for establishing the true prevalence of celiac disease in Israel [5].

The complexity of the clinical presentation of this disease necessitates a wide use of serologic tests in clinical practice by primary physicians and gastroenterologists. The laboratory serving one of the main health providers in Israel (Maccabi Healthcare Services) has a cost-effective screening policy for celiac disease: namely, measurement of total IgA and tTGA followed by confirmation of positive results by EMA. The aim of the present study, initiated by the laboratory, was to evaluate its own policy - namely, the use of tTGA as the initial screening test and EMA as a confirmatory test in symptomatic patients.

Ig = immunoglobulin

tTGa = transglutaminase IgA antibodies

EMA = endomysial antibodies

tTG = tissue transglutaminase

Materials and Methods

Serologic tests for celiac disease in Maccabi Healthcare Services are performed by one national central laboratory that receives samples from all over the country. The results of tTGA and EMA tests over a 3 month period (1 July to 30 September 2001) were obtained from the laboratory database. Tests were identified by ID number only. When tTGA tests were positive, a letter was sent to the referring physician with a request for information including: age, gender, ethnicity (Ashkenazi, Sephardic*), clinical symptoms leading to the serologic screening, and small intestinal biopsy results, if performed.

IgA antibodies against tTG were measured using a commercial ELISA (Immco, USA), based on guinea pig tissue transglutaminase as a substrate. The test was quantitative and values were obtained in units/ml (U/ml). Values greater than 20 U/ml were considered positive for IgA tTG antibodies as established by the manufacturer. EMA was analyzed by indirect immunofluorescence assay (Immco) according to the manufacturer's instructions. The serum concentration of IgA was measured by nephelometry (Dade-Behring, Germany).

Intestinal biopsy samples were obtained from the duodenum during upper gastrointestinal endoscopy. Although the biopsies were performed and examined by different pathologists in several hospitals in Israel, the diagnosis was based on established criteria [18].

Statistical analysis

Categorical variables were compared using Fisher's exact test and continuous variables by Students' *t*-test. The positive predictive value of tTGA for celiac disease was calculated as the ratio between the number of patients with the disease and positive tTGA and the sum of all subjects with positive tTGA who underwent intestinal biopsies [19].

Results

During the 3 month period the laboratory performed 2,505 tTGA tests. Of these, 216 samples were tTGA-positive (8.6%): 162 of them (75%) were EMA-negative (group 1) and 54 (25%) were EMA-positive (group 2). Clinical and histologic information was obtained for 91 patients (56%) in group 1 and 32 (59%) in group 2.

The clinical characteristics of patients are presented in Table 1. The geographic distribution of patients comprised Beer Sheva in the south to Netanya in the center of the country, and from the Jerusalem and Samaria areas. Age, gender and ethnic distribution were similar in both groups. The symptoms leading to serologic screening for celiac disease included various gastrointestinal complaints, growth failure, iron deficiency anemia, family history of celiac disease, and related autoimmune diseases. There was no difference between the two groups regarding patients' characteristics or the occurrence of symptoms.

Small intestinal biopsy was performed in 33 patients (36%) in group 1 and 27 (84%) in group 2 ($P < 0.0001$). Celiac disease was

Table 1. Demographic and clinical characteristics of the study groups

	Group 1* (n=91)	Group 2** (n=32)
Age (yrs)	17.9 ± 11.2	18.2 ± 15.1
Gender		
Males	39	12
Females	52	20
Ethnicity		
Ashkenazi	50	19
Sephardic	41	13
Symptoms: No. (%) of patients		
Abdominal pain	15 (16.5%)	5 (15.6%)
Diarrhea	15 (16.5%)	6 (18.7%)
Weight loss	8 (8.8%)	4 (12.5%)
Short stature	12 (13.2%)	7 (21.8%)
Anemia	14 (15.4%)	5 (15.6%)
Type 1 diabetes	6 (6.6%)	2 (6.2%)
Autoimmune disease	2 (2.2%)	2 (6.2%)
Family history of celiac disease	3 (3.3%)	3 (9.4%)
Other	15 (16.5%)	3 (9.4%)

* Group 1: tTGA-positive/EMA-negative

** Group 2: tTGA-positive/EMA-positive

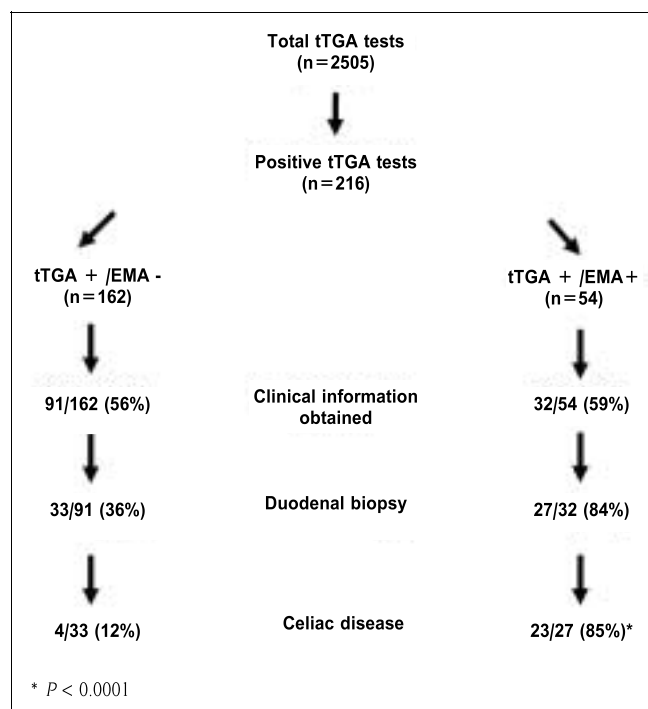


Figure 1. Summary of screening symptomatic patients with first-line tTGA test diagnosed in 4 of 33 biopsies (12%) in group 1 and in 23 of 27 (85%) in group 2 ($P < 0.0001$). The positive predictive value of the tTGA test for celiac disease is 45% compared to 85% for EMA. The results are summarized in Figure 1.

In group 1, two patients had duodenitis and 27 patients had normal small intestinal biopsies. One of them had a first-degree relative with the disease and may represent latent disease. No other risk factors for celiac disease were present in the other 26 patients. In group 2, four patients had normal biopsies: two had first-degree

* Ashkenazi refers to East European origin, and Sephardic to North African or Middle East origin.

ELISA = enzyme-linked immunosorbent assay

relatives with the disease, one had type 1 diabetes and one had hypothyroidism. These patients may also have latent celiac disease.

Discussion

The diversity of celiac disease and its potential complications indicate the necessity for non-invasive screening to identify and treat unrecognized patients [20,21]. While celiac disease-related serology (EMA and tTGA) is suitable for screening the general population, several problems have yet to be resolved. These include: estimating the number of missed new cases, devising the most reliable screening algorithm, and developing the most cost-effective analysis. In Israel, each healthcare provider uses a different combination of serologic tests for screening and the referring physicians have to choose from available tests. Our study addressed one aspect of screening – the use of tTGA as the first step and EMA as a confirmatory serologic test in a population of patients with gastrointestinal symptoms or risk factors for the disease. The main finding of our study was that a positive tTGA test requires confirmation by EMA, since in tTGA-positive/EMA-negative patients the rate of the disease was only 12%, compared to 85% in tTGA-positive/EMA-positive patients.

Gomez et al. [22] recently compared the screening value of two different protocols for celiac disease in the same community-based populations: a three-level screening using IgG and IgA antigliadin antibodies as the first level, followed by EMA and intestinal biopsy of positive patients, compared to tTGA as the first step (commercial guinea-pig anti-tTG antibody), EMA for positive patients only and intestinal biopsy. The second protocol was superior both in identifying subjects and in cost-effectiveness. Using the second protocol, which is identical to the protocol in our study, six patients had tTGA-positive/EMA-negative results; intestinal biopsies performed in two of them revealed a normal mucosa. On the other hand, six patients positive for both tTGA and EMA were found to have the disease. The authors concluded that a screening algorithm based on the combination of the highly sensitive anti-tTG antibodies as the first level and the specific EMA as a second test might be optimal for the screening, avoiding unnecessary biopsies. Dickey et al. [17], in a study of active celiac disease patients, found that a third of them had only one antibody – either tTGA or EMA – and recommended using combined screening.

The sensitivity and specificity of tTGA for celiac disease are high and similar to those of EMA [11–15]. In a recent study of blood donors in Israel, Shamir and co-workers [5] found low sensitivity and specificity for celiac disease when one serologic marker was used: 50% and 38% for tTGA, and 70% and 68% for EMA, respectively. The positive predictive value was 33% for tTGA and 54% for EMA. The study population was mostly asymptomatic. Hoffenberg et al. [23] investigated the positive predictive value of tTG antibodies in asymptomatic children at genetic risk for the disease and found it to be 70–83%. In our study, screening was performed in subjects with gastrointestinal symptoms or risk factors for the disease. The sensitivity and specificity of tTGA was not defined since no uniform information about tTGA-negative patients could be obtained. The positive predictive value of tTGA was 45%, higher than reported by Shamir et al., but significantly lower than

the 85% of EMA. The prevalence of celiac disease in Israel was estimated in a single small-scale study as at least 1:157 [5], however the exact prevalence is unknown. Therefore, we calculated the positive predictive value as the ratio between the number of patients with the disease and positive tTGA and the sum of all subjects with positive tTGA who underwent intestinal biopsies [19].

Several anti-tTGA commercial kits are available, containing either guinea pig or human tTG. The human tTGA kit was found to have the highest sensitivity and specificity [24]. It is possible that an anti-human tTGA test could improve the performance of the test.

A shortcoming of our study is the lack of a control group of EMA-positive patients as a first-line test. Such a group was not available for the study since it is not the current policy of the laboratory. In a previous study [25], the laboratory screened 12,472 sera samples for tTGA and EMA for comparison of assay sensitivity, and EMA did not show superior sensitivity over tTGA. However, no clinical or histologic correlation was performed. In the present study clinical information was available in only 56–59% of the patients, and a larger number may have given a more accurate background of the patients. Nonetheless, the proportion of patients is similar in both study groups and therefore the data gathered appropriately represent the actual rate of diagnosis in the whole study group.

In conclusion, our study shows that confirmation with EMA is needed when tTGA is performed as a first-level screening in patients suspected of having celiac disease. A tTGA positive/EMA-negative result predicts a low probability of disease. The appropriate approach to such patients is not clear and warrants further studies.

References

- Cattassi C. Screening of celiac disease. In: Maki M, Collin P, Visakorpi JK, eds. Celiac Disease. Proceedings of the 7th International Symposium on Celiac Disease. Tampere: Celiac Disease Study Group, 1997:23–33.
- Logan RFA. Screening for coeliac disease: has the time come for mass screening? *Acta Paediatr* 1996;85(Suppl 412):15–19.
- Collin P, Kaukinen K. Clinical news: the occurrence and treatment of celiac disease. In: Auricchio S, Greco L, Maiuri L, Troncone R, eds. Celiac Disease. Proceedings of the 8th International Symposium on Celiac Disease. Naples: JCG Editions, 2000:119–23.
- Fasano A, Cattassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology* 2001;120:636–51.
- Shamir R, Lerner A, Shinar E, et al. The use of a single serological marker underestimates the prevalence of celiac disease in Israel: a study of blood donors. *Am J Gastroenterol* 2002;97:2589–94.
- Ascher H. The role of quantity and quality of gluten-containing cereals in the epidemiology of celiac disease. In: Maki M, Collin P, Visakorpi JK, eds. Celiac disease. Proceedings of the 7th International Symposium on Celiac Disease. Tampere: Celiac Disease Study Group, 1997:15–22.
- Corrao G, Corazza GR, Andreani ML, et al. Serological screening for coeliac disease: choosing the optimal procedure according to various prevalence values. *Gut* 1994;35:771–5.
- Johnston SD, Watson RGP, Mcmillan SA, Sloan J, Love AH. Coeliac disease detected by screening is not silent – simply unrecognised. *Q J Med* 1998;91:853–60.
- Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BME, Meijer JWR, Mulder CJJ. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. *Am J Gastroenterol* 1999;94:888–94.
- Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997;3:797–801.

11. Vitoria JC, Arrieta A, Arranz C, et al. Antibodies to antigliadin, endomysium and tissue transglutaminase for the diagnosis of celiac disease. *J Pediatr Gastroenterol Nutr* 1999;29:571–4.
12. Biagi F, Ellis HJ, Yiannakou JY. Tissue transglutaminase antibodies in celiac disease. *Am J Gastroenterol* 1999;94:2187–92.
13. Chan AW, Butzer JD, McKenna R, Fritzlner MJ. Tissue transglutaminase enzyme-linked immunosorbent assay as a screening test for celiac disease in pediatric patients. *Pediatrics* 2001;107:E8.
14. Gillett HR, Freeman HJ. Comparison of IgA endomysium antibody and IgA tissue transglutaminase antibody in celiac disease. *Can J Gastroenterol* 2000;14:668–71.
15. Sulkanen S, Halttunen T, Laurila K, et al. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology* 1998;115:1322–8.
16. Sugai E, Selvaggio G, Vazquez H, et al. Tissue transglutaminase antibodies in celiac disease: assessment of a commercial kit. *Am J Gastroenterol* 2000;95:2318–22.
17. Dickey W, McMillan SA, Hughes DF. Sensitivity of serum tissue transglutaminase antibodies for endomysial antibody positive and negative coeliac disease. *Scand J Gastroenterol* 2001;36:511–14.
18. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity (celiac sprue). *Gastroenterology* 1992;102:330–54.
19. Fletcher RH, Fletcher SW, Wagner EH. Diagnosis. In: Fletcher RH, Fletcher SW, Wagner EH, eds. *Clinical Epidemiology – The Essentials*. 3rd edn. Baltimore: Williams & Wilkins, 1996:43–74.
20. Not T, Hovarth K, Hill ID, et al. Coeliac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors. *Scand J Gastroenterol* 1998;33:494–8.
21. Collin P, Reunala T, Ransmussen M, et al. High incidence and prevalence of adult coeliac disease. *Scand J Gastroenterol* 1997;32:1129–34.
22. Gomez JC, Selvaggio G, Pizarro B, et al. Value of a screening algorithm for celiac disease using tissue transglutaminase antibodies as first level in a population-based study. *Am J Gastroenterol* 2002;97:2785–90.
23. Hoffenberg EJ, Bao F, Eisenbarth GS, et al. Transglutaminase antibodies in children with a genetic risk for celiac disease. *J Pediatr* 2000;137:356–60.
24. Hansson T, Dahlbom I, Hall J, et al. Antibody reactivity against human and guinea pig tissue transglutaminase in children with celiac disease. *J Pediatr Gastroenterol Nutr* 2000;30:379–84.
25. Parizada M, Shainberg B. Tissue transglutaminase and endomysial antibodies, similar but not identical serological markers of celiac disease [Abstract]. *Autoimmun Rev* 2002;1:90.

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