

Short communication

Comparison of ELISA for tissue transglutaminase autoantibodies with antiendomysium antibodies in pediatric and adult patients with celiac disease

Background: Tissue transglutaminase (t-TG) is the main autoantigen recognized by the endomysium antibodies (EMA) observed in patients with celiac disease (CD). The aim of the study was to assess an ELISA method for t-TG antibodies (t-TGA) with respect to EMA IF assay in pediatric and adult patients.

Methods: t-TGA were analyzed by ELISA in 220 sera samples: 82 patients with biopsy-proven untreated CD (23 adults and 59 children), 14 CD children on gluten-free diet, 18 asymptomatic relatives of CD patients, and 106 age-matched control patients with gluten-unrelated gastrointestinal diseases (58 adults and 48 children). Serum IgA EMA were tested on umbilical cord sections in all patients.

Results: The great majority (92.7%) of untreated CD patients (both adults and children) were t-TGA positive (values ranging from 20.1 to >300 AU). None of the child control patients and only two out of 58 (3.4%) of the adults with unrelated gastrointestinal diseases had serum t-TGA positivity; two out of 18 first-degree relatives with biopsy-proved silent CD were t-TGA (as well as EMA) positive. Finally, two out of 14 CD children, assuming a gluten-free diet, had serum t-TGA (as well as EMA). A highly significant correlation ($P < 0.001$) was observed between t-TGA concentrations and EMA. t-TGA showed a sensitivity of 87% and 95%, a specificity of 97% and 100% for adults and children, respectively.

Conclusions: The method is highly sensitive and specific in the diagnosis of CD and is promising as a tool for routine diagnostic use and population screening, especially in children.

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Key words: celiac disease; ELISA; endomysium antibodies; tissue transglutaminase antibodies.

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Accepted for publication 12 January 2001

Gluten hypersensitivity, or celiac disease and related syndromes, is becoming a significant health problem. Recent European surveys have demonstrated that the true prevalence of celiac disease (CD) is higher than previously thought, approaching 1:180 in some countries (1). In most cases, the clinical diagnosis is underestimated, since most affected individuals do not display an overt classical malabsorption syndrome, being symptomless for a long time or having unexplained mild iron or calcium deficiencies.

Since the discovery of the immunologic basis of CD, the search for specific antibodies has been considered an important diagnostic tool. Endomysium antibodies (EMA) assays by indirect immunofluorescence (IF) are currently regarded as the most sensitive and specific immunologic marker of CD (2–5). The problem of its higher cost with respect to antigliadin antibodies has been, at least in part, overcome with the use of human umbilical cord as a substrate, instead of monkey esophagus (4, 5).

Recently, the major, if not the sole, autoantigen responsible for endomysium reactivity in CD has been identified as tissue transglutaminase (6). This enzyme has great affinity for gliadin and is able to modify it, thus increasing its affinity for DQ2-restricted cytotoxic T cells, which are probably responsible for typical gut mucosal damage (7).

Since this discovery, several immunometric assays for t-TG antibodies (t-TGA) have been developed, in an attempt to improve the diagnostic performance of EMA antibodies, or at least to set up a more easy-to-use diagnostic tool with respect to EMA IF assay. This may be important when large numbers of samples have to be processed. The results reported of t-TGA immunoassays are in general satisfactory; however, sensitivities ranging from 85% to 100% and specificities from 90% to 99.5% have been reported, only in part dependent on the source of autoantigen (whether purified from guinea-pig liver or human recombinant) (8–23).

In this study, we present the results of an original ELISA method for detection of t-TGA in the serum, and compare the results obtained with EMA IF in a large group of CD patients and diseased controls. Unlike other studies, pediatric and adult patients were separately considered with appropriate age-matched controls.

Material and methods

The study population comprised 220 subjects. Serum samples from 82 patients with untreated CD consecutively selected between 1996 and 1999 were studied (23 were adults: age range 27–96 years, median 50 years, 13 female and 10 male; 59 were children: age range 2–14 years, median 8.2 years, 22 male, 37 female); CD diagnosis was based on duodenal biopsy (grades I–IV villous atrophy, according to Marsh's criteria) (24) and response to a gluten-free diet. Gluten challenge was not systematically performed in children, and the outcome of immunologic markers after a gluten-free diet was not available for all the patients. Age-matched patients with gluten-unrelated gastrointestinal diseases proved by a biopsy (58 adults and 48 children) were used as controls. Fourteen CD child patients on a gluten-free diet for at least 6 months and 18 asymptomatic relatives of CD patients were also analysed. Only patients with normal IgA levels were included in the study (IgA cutoff 22–80 mg/dl depending on age).

Serum IgA EMA were tested by IF on umbilical cord sections as previously described (minor modifications with respect to Ladinsler's method) (4).

For t-TGA ELISA, flat-bottomed, 96-well microplates (Nunc, Roskilde, Denmark) from a single lot were coated with transglutaminase from guinea-pig liver (Sigma cat. T-5398) at the concentration of 10 µg/ml in 0.1 M phosphate-buffered saline (PBS), pH 7.5.

Serum samples were diluted 1:200 in PBS-0.1% BSA: the optimal dilution was assessed on the basis of preliminary titration experiments. Volumes of 100 µl of serum diluted samples were dispensed in t-TGA-coated microwells and incubated for 1 h at room temperature. After extensive washing in PBS-BSA, an affinity-purified polyclonal antihuman IgA antibody labeled with peroxidase (Kirkegaard & Perry, cat. 14-10-01) at the dilution of 1:3000 in Tris buffer was added, and incubation was carried out for 30 min at room temperature. The microplates were then washed, and the chromogen (tetramethylbenzidine, TMB 20 µg/well) was added. The reaction was stopped after 10 min by adding H₂SO₄. Parallel samples containing different dilutions of a reference pool of sera containing high t-TGA concentration, corresponding to 0, 30, 150, and 300 arbitrary units (AU), were set up. Colorimetric reading was performed at 450 nm.

Results

The performance of the ELISA for t-TGA we set up proved to be satisfactory. The cutoff point was established by ROC curves analysis (25), from the data obtained for untreated CD and patients with gluten-unrelated gastrointestinal diseases; overall data analysis is depicted in Fig. 1. Separate analyses were also performed for pediatric and adult subjects with superimposable results (data not shown). The optimal cutoff was found to be 20 AU for both adults and children. The intra-assay coefficients of variation, as calculated from 10 replicates of three sera at the

concentrations of 47, 187, and 267 AU ranged from 5.5% to 9.7%, and the between-assay coefficients of variation ranged from 5.6% to 17%.

The results of the t-TGA assay in the different groups of patients studied are shown in Fig. 2. As clearly shown, the great majority (92.7%; 95% confidence limits 84.75–97.27) of untreated CD patients (both adults and children) had t-TGA in their sera, at concentrations ranging from 20.1 to >300 AU; a sensitivity of 87% and 95% for adults and children, respectively, was observed. The specificity of the test was very good, as none of child patients, and only two out of 58 (3.4%) of the adults with gluten-unrelated gastrointestinal diseases had serum t-TGA positivity, (specificity of 97% and 100% for adults and children, respectively). Among CD patients' first-degree relatives, two out of 18 were t-TGA (as well as EMA) positive; gut biopsy revealed II and III degree villous atrophy, indicating that these subjects, although symptomless, had silent CD. Finally, two out of 14 CD children on a gluten-free diet had serum t-TGA (209.6 and 21.6 AU, respectively), as well as EMA. There was no correlation between EMA or t-TGA and the degree of villous atrophy, as previously observed for EMA (5).

A highly significant correlation ($P < 0.001$, Spearman's test) was observed between t-TGA concentrations and EMA titers (from 1:5 to 1:400). A comparative plot of EMA vs t-TGA in CD patients (untreated and on gluten-free diet) is shown in Fig. 3. Despite the close correlation observed, t-TGA appeared to be a slightly less sensitive marker than EMA (four out of 82 untreated CD patients were EMA-positive and t-TGA-negative, and only one adult CD was EMA-negative and slightly t-TGA positive). "Nonspecific" t-TGA positivity

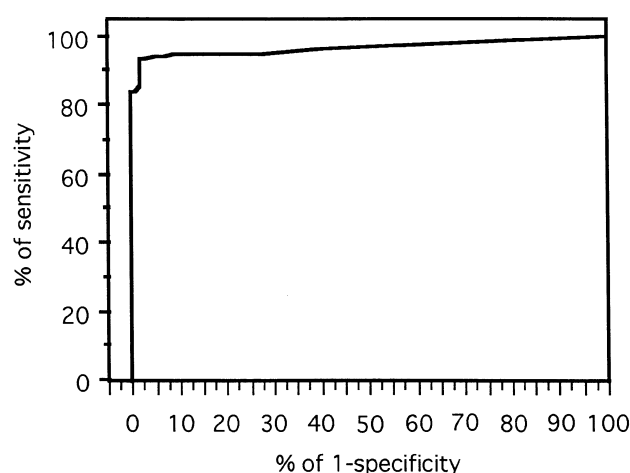


Figure 1. ROC curves analysis obtained for untreated CD (adults and children) and patients with gluten-unrelated gastrointestinal disease.

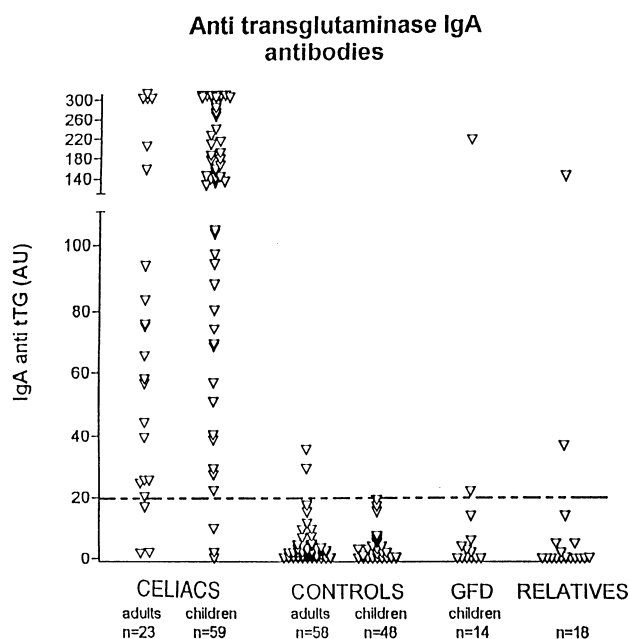


Figure 2. Levels of transglutaminase antibodies in celiacs, diseased controls, and relatives of celiac patients. Dashed line represents cutoff limits (= 20 AU). GFD: CD children on gluten-free diet.

was observed in two adult diseased controls, who were EMA-negative. The EMA IF assay proved to have sensitivities of 87% and 100% for adults and children, respectively, and a specificity of 100% for both adults and children.

Discussion

The results of our study indicate the good sensitivity and specificity of our t-TGA assay for the diagnosis of CD, for both adults and children. The test displayed a slightly better performance in children than in adults. The overall sensitivities and specificities were very similar to others reported in recent literature (8–23). In such studies, sensitivity ranged from 85% to 100% and specificity from 90% to 99.5%; note that the specificity was not markedly affected by the type of control population chosen (healthy blood donors or diseased controls) (19, 20, 22). The use of human recombinant tissue transglutaminase as antigen for the t-TGA assay has been proposed to improve sensitivity (17, 18, 21).

The agreement with EMA IF results was excellent, the quantitation of t-TGA exactly correlating with EMA titers. This once again confirmed that t-TG is the main autoantigen causing EMA reactivity. However, on the basis of the present results, the sensitivity of t-TGA ELISA was slightly less than that of EMA IF in children. Note that EMA sensitivity and specificity in our study are superimposable on

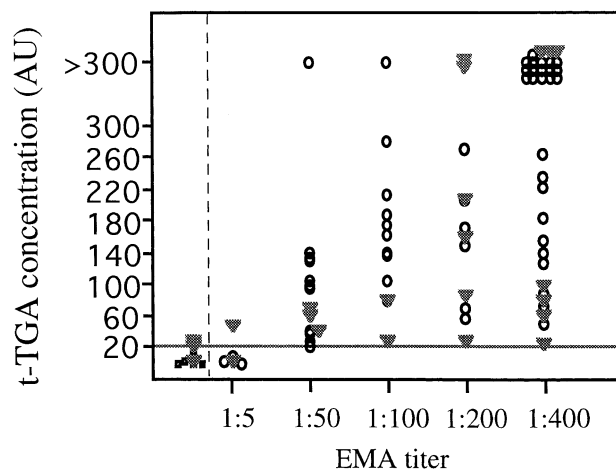


Figure 3. t-TGA vs EMA in patients with celiac disease. ○: children with untreated celiac disease; □: children with celiac disease on gluten-free diet; ▼: adults with untreated celiac disease; —: TGA cutoff point (20 AU); —: EMA cutoff point (<1:5).

those of other studies (2, 3). Moreover, a slight relative loss of specificity of t-TGA as compared to EMA has been observed, notably in adults. One cannot rule out the possibility that other minor autoantigen specificities contribute to EMA reactivity. However, the overall performance of our quantitative t-TGA immunoassay was good, and the implementation of full automation may render it more useful for diagnosis and population screening of celiac disease than IF methods when large numbers of samples have to be processed. Comparative “prospective” studies (i.e., on newly diagnosed patients from at-risk groups) are obviously needed to determine the diagnostic values of EMA IF and t-TGA ELISA in the early diagnosis of CD. In addition, it remains to be ascertained whether immunoassay of t-TG IgG antibodies may have other diagnostic value, especially to identify IgA-deficient CD patients. Finally, both t-TGA and EMA were present in two out of 14 CD patients on a gluten-free diet: this may indicate similar sensitivities in detecting poor compliance to the gluten-free diet, although confirmation with a larger sample is needed.

Acknowledgments

We thank the technical staff of the Laboratory of Immunology, Université Libre de Bruxelles, and Mrs E. Borini (DIMI, Genoa University) for technical support. We are indebted to Dr Y. Vandenplas (Department of Pediatrics Academisch Ziekenhuis Kinderen Free University of Brussels, Belgium) and to E. Castellano (III Pediatric Division, Istituto G. Gaslini, Genoa, Italy) for providing the sera used in this study. We also thank Dr G. Gallucci (Radim, S.p.A., Italy) for helpful discussion. This work was partially supported by grants from Italian MURST.

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