ELISA of Anti-Endomysial Antibodies in the Diagnosis of Celiac Disease: Comparison with Immunofluorescence Assay of Anti-Endomysial Antibodies and Tissue Transglutaminase Antibodies

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Key words: celiac disease, anti-endomysial antibodies, tissue transglutaminase antibodies, enzyme-linked immunosorbent assay, immunofluorescence

Abstract

Background: Celiac disease is common in both children and adults. Small intestinal biopsy is mandatory for establishing a diagnosis. Anti-endomysial antibodies, detected by immunofluorescence, have a sensitivity and specificity close to 100% in the diagnosis of CD. Recently, tissue transglutaminase has been identified as the target autoantigen of antibodies against endomysium, and TTG antibodies are comparable to EMA-IMF in the diagnosis of CD.

Objective: To evaluate a new enzyme-linked immunosorbent assay kit for EMA, compared to EMA-IMF and TTG antibodies in the diagnosis of CD.

Methods: Our study population included all subjects with positive EMA-IMF who underwent intestinal biopsy (n=21). From the same sera, TTG antibodies and EMA-ELISA were determined, and all antibody results were compared to the biopsy findings.

Results: EMA-IMF was able to predict biopsy findings of CD in 19 of 21 cases (90.5%). When patients with biopsy findings compatible with CD and positive EMA-IMF (n=19) were tested for EMA-ELISA and TTG antibodies, 18 of the 19 were positive for both EMA-ELISA and TTG antibodies. A significant correlation was found between EMA-ELISA and TTG antibody titers ($r = 0.74$, $P < 0.001$).

Conclusions: Our study demonstrates that EMA-ELISA is comparable to TTG antibodies in the diagnosis of CD, and supports the use of EMA-ELISA as a serologic marker for this disease.

IMAJ 2002;4:594–596

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Celiac disease is common in both children and adults, and is characterized by abnormal small intestinal mucosa, permanent intolerance to gluten, and full recovery of clinical, biochemical and histologic findings on a gluten-free diet [1].

Small intestinal biopsy is mandatory for establishing the diagnosis of CD, but the diagnosis of CD is complicated by the fact that the disease may be silent or may be present with atypical findings [2–5]. Therefore, serologic markers are used both to select patients requiring biopsy and to monitor response and adherence to a gluten-free diet.

Anti-endomysial antibodies have a sensitivity and specificity of close to 100% in the diagnosis of CD in individuals without immunoglobulin A deficiency, and are considered superior to IgA-antigliadin antibodies for diagnosing individuals with CD [6,7]. They are also the preferred serologic marker in population screening [8,9]. EMA testing has a few but important drawbacks: the immunofluorescence test is technically difficult to interpret with large inter-observer variability, it uses esophageal tissue from monkeys, and it is time consuming which is a burden for laboratories performing mass screening.

Tissue transglutaminase was recently found to be the autoantigen recognized by EMA [10]. Tissue transglutaminase antibodies are also highly sensitive and specific [7,11–15], and since IgA antibodies to TTG are examined by ELISA, they are easier to use as screening antibodies as compared to the EMA testing. However, there is an incomplete overlap between EMA testing and commercial TTG antibody kits [14]. Additional antigens on the endomysium as well as species differences may account for these differences. Indeed, human-based TTG antibodies were recently found to be more sensitive than guinea pig-based TTG antibodies, although their specificity was similar [15].

The aim of our study was to evaluate a new ELISA assay for EMA (EMA-ELISA), compared to EMA-IMF and TTG antibodies in the diagnosis of CD, and to determine whether quantitative EMA testing correlates with biopsy findings.

Patients and Methods

Study population

We retrieved all positive EMA-IMF sera tested in our laboratory between January 2000 and January 2001 (n=30). The local ethics committee approved the study protocol. The study population included all subjects with positive EMA-IMF who, after EMA-IMF testing, underwent intestinal biopsy that was suggestive of CD (n=21). The study group comprised 11 children (5 females) aged 2–19 years and 10 adults (4 females) aged 20–55. TTG and EMA-ELISA

CD = celiac disease
TTG = tissue transglutaminase
EMA-IMF = anti-endomysial antibodies measured by immunofluorescence
ELISA = enzyme-linked immunosorbent assay

IG = immunoglobulin

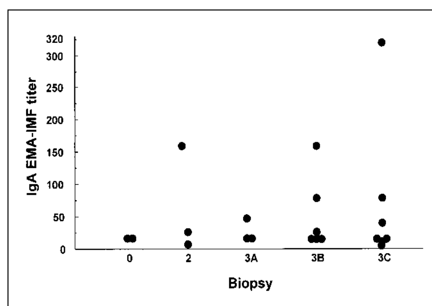

Figure 1. Distribution of EMA-IMF titers and biopsy findings, showing levels of antibody titers for EMA-IMF. Values are given separately for each Marsh classification.

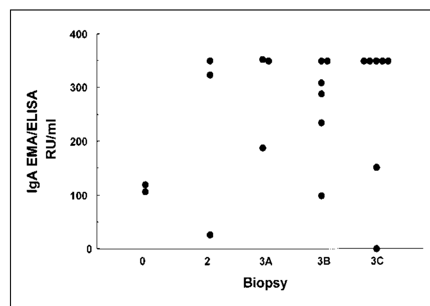

Figure 2. Distribution of EMA-ELISA titers and biopsy findings. Values are given in RU/ml of IgA antibodies to EMA measured by ELISA. Values are given separately for each Marsh classification; the range of positive values was 25.8–350 RU/ml.

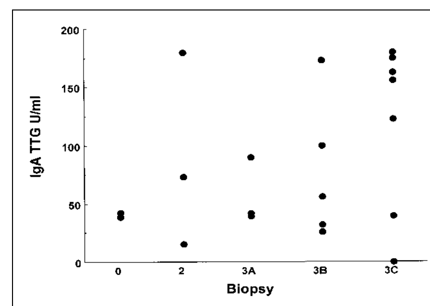

Figure 3. Distribution of TTG titers and biopsy findings. Values are given in U/ml of IgA antibodies to TTG measured by ELISA. Values are given separately for each Marsh classification; the range of positive values was 16–180 U/ml.

were determined from the same sera, and all antibodies were compared with biopsy findings and grading.

EMA-IMF evaluation

IgA EMA was analyzed by indirect immunofluorescence microscopy using fixed cryostat sections of monkey esophagus (The Binding Site, Birmingham, UK) as the antigen substrate, as previously described [16]. All sera were diluted at 1:2.5, and positive samples were further diluted to the highest dilution yielding fluorescence.

EMA-ELISA evaluation

IgA EMA was evaluated by ELISA using a commercial kit (Euroimmun, Germany). The EMA-ELISA is based on endomysium antigens purified by affinity chromatography from primate liver. Briefly, the sera for analysis were diluted 1:200 with ready-to-use sample buffer, while calibration and control sera were pre-diluted and ready to use. Following the ELISA procedure, photometric measurement of the color intensity was done at a wavelength of 450 nm with a reference wavelength of 620 nm. Since no international reference serum exists for endomysial antibodies, the calibration was performed in relative units. The manufacturer-defined cut-off level was 20 RU/ml. Values above the cut-off were considered positive and values below were considered negative. Both positive and negative control sera served as internal controls for the reliability of the procedure. The standard curve, from which the concentration of the antibodies was taken, was obtained by point-to-point plotting of the extinction values measured for the three calibration sera supplied against the corresponding units.

TTG antibodies evaluation

IgA antibodies against TTG were measured using a commercial ELISA assay (Orgentec Diagnostika, Germany) based on recombinant human TTG as an antigen. The test was quantitative, and values were obtained in units/ml. Sera were diluted 1:100, or to higher dilutions when positive results were obtained, and the optical density was determined at 450 nm with a reference of 620 nm. Values above 15 U/ml were considered positive for IgA TTG antibodies as established by the manufacturer.

RU = relative units

Intestinal biopsy

Biopsies were evaluated using the Marsh criteria [17] with the modification published by Rostami et al. [18]. Briefly, Marsh 1 is characterized by normal architecture of the villi with marked lymphocytic infiltration, Marsh 2 by a hyperplastic lesion with crypt elongation, Marsh 3A by the addition of shortened and blunted villi, Marsh 3B by the presence of villi that are clearly atrophic but still present, and Marsh 3C by total absence of villi with severe atrophy, hyperplasia and infiltration.

Statistical analysis

Linear regression was used to compare biopsy findings with antibody titers. Pearson correlation coefficients were calculated for the associations between antibody titers.

Results

Histologic evaluation of the 21 biopsies revealed normal small intestinal mucosa in 2 biopsies, hyperplastic lesion (Marsh 2) in 3 biopsies and partial to total villous atrophy in 16 (Marsh 3B and Marsh 3C).

The distribution of antibodies according to biopsy grading for EMA-IMF is shown in Figure 1, for EMA-ELISA in Figure 2, and for TTG antibodies in Figure 3. EMA-IMF was able to predict biopsy findings of CD in 90.5% of cases. The two patients with normal biopsies were positive to all three antibodies tested. When patients with biopsy findings compatible with CD and positive EMA-IMF (n=19) were tested for EMA-ELISA and TTG antibodies, 18 of 19 were positive for both EMA-ELISA and TTG antibodies. One positive EMA-IMF with biopsy compatible with CD tested negative to both EMA-ELISA and TTG antibodies.

Using linear regression, no correlation was found between the severity of biopsy findings and antibody titer. However, a significant correlation was found between EMA-ELISA and TTG antibodies (r = 0.74, $P < 0.001$).

Discussion

Our study results demonstrate a high correlation between the new EMA-ELISA assay and the human TTG antibodies, similar to the high correlation previously found between TTG antibodies and EMA-IMF [7,11–13,19]. EMA-ELISA was positive in all CD patients

who were positive for TTG antibodies, and both ELISA methods were negative in one CD patient who was EMA-IMF-positive. These results suggest that EMA-ELISA is as good as human TTG antibodies in predicting biopsy findings, and are in agreement with previous suggestions that EMA-IMF may be a better serologic marker for CD than TTG antibody [11,14].

Two cases that were positive for all three serologic markers had a normal biopsy. Although these cases may turn out to be latent CD, the results demonstrate a 9.5% false positive rate for EMA-IMF and a 14.3% false positive rate for EMA-ELISA and TTG antibody. The false positive rate for EMA-IMF is similar to that found in previous studies [19–21], but is in contrast to other studies demonstrating a complete agreement between EMA-IMF and histopathologic findings [22,23]. Our study did not find a significant correlation between biopsy findings and antibody titers of EMA-IMF, EMA-ELISA or TTG antibodies. The lack of correlation between antibody titer and biopsy findings, and the discrepancy in the percentage of false positivity for EMA-IMF in different studies may result from the tendency of EMA antibodies to have a high predictive value only in the presence of severe mucosal lesion [24,25]. Thus, the false positivity rate will drop as the percentage of cases with severe mucosal lesion increases. Since our study design prompted us to examine biopsy findings only in EMA-IMF-positive patients, the usefulness of EMA-IMF in mild mucosal lesion could not be tested. Nevertheless, a severe mucosal lesion was found only in 16 of the 21 cases in our study, while in a previous study all patients with EMA-IMF had severe lesion [25].

One should keep in mind that in our study the sample rize was small and since none of the cases were EMA-IMF-negative, we could not compare the sensitivity and specificity of the different serologic markers. Nevertheless, in 19 cases diagnosed with CD, EMA-ELISA and TTG antibodies were useful in 94.7% of cases in the diagnosis of CD.

In conclusion, our study demonstrates that EMA-ELISA is comparable to TTG antibodies in the diagnosis of CD, and supports the use of EMA-ELISA as a serologic marker for this disease.

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