Autoantibodies to tissue transglutaminase in children with celiac disease: comparison of guinea pig and human transglutaminase antigens

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Abstract

Tissue transglutaminase (tTG) has been identified as a main antigen of endomysial and antireticulin antibodies found in celiac disease (CD). To assess the diagnostic validity of serum anti-tTG antibody levels, commercial enzymatic linked immunosorbent assays (ELISA) with human recombinant (h) and guinea pig (Gp) tTG were used. IgA anti-tTG antibodies were determined in 50 sera of patients with untreated CD, 55 sera of patients on gluten free diet (GFD) and 50 sera of non-celiac controls. Results were correlated with clinical and histological data. Anti-hTG antibodies were found in 47 patients of untreated CD group (sensitivity of the test 94%) and anti-GptTG in 46 (sensitivity 92%). All of controls were negative for hTG antibodies (specificity of the test 100%), whereas 6 control patients were positive for Gp-tTG antibodies (specificity 90%). The level of antibodies did not correlate with histological changes of jejunal mucosa, but the amount of both antibodies decreased in patients on GFD. Our results revealed that hTG assay is a better tool than Gp-tTG one for diagnosis and screening programs of CD.

Key words: anti-guinea pig tissue transglutaminase antibodies (Gp-tTG Ab), anti-human tissue transglutaminase antibodies (hTG Ab) • celiac disease

Introduction

Celiac disease (CD) is a life long disorder recognized to be an immunologically mediated enteropathy of small intestine, occurring in genetically susceptible individuals after ingestion of gluten [15]. The diagnosis of CD is established by the histological demonstration of villous atrophy, crypt hyperplasia, increased number of intraepithelial lymphocytes and inflammation in lamina propria, followed by clinical remission after gluten withdrawal from the diet [13]. For long time three main categories of circulating antibodies, i.e. anti-gliadin, anti-endomysial and anti-reticulin have been identified as having value in diagnosis and screening of CD. In 1997 Dieterich et al. identified tissue transglutaminase (tTG) as the main endomysial autoantigen [6], and in 2000 Korponay-Szaso et al. showed that tTG was the antigen for antireticulin antibodies [12]. tTG belongs to the family of transglutaminase enzymes that catalyze the acyl-transfer reaction in which â-carboxamide groups of peptide – bound glutamine residues serve as acyl donors. This action results in the formation of isopeptide bounds between glutamine (Gln) and lysine (Lys) residues. Gliadins are rich in Gln residues, so can be accepted as the substrate for tTG. It was demonstrated that tTG deamidates gliadin peptides and in that way creates epitopes that bind to DQ2 or DQ8 receptors of gut-derived T cells inducing their activation [14]. These activated T cell clones are responsible for production of proinflammatory cytokines that effect mucosa [15].

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The discovery of tTG as the autoantigen, on the one hand improved our knowledge on pathogenesis of CD, on the other led to development of enzymatic linked immunosorbent assays (ELISA) that detect tTG antibodies. Many studies indicate good correlation between anti-tTG and anti-endomysial antibodies, however there are discrepancies concerning the specificity and sensitivity of anti-tTG antibodies [2, 4, 5, 7, 9]. The first generation of tests for IgA tTG antibodies based on commercially available guinea pig tTG (GptTG) showed sensitivity 89-100%, and specificity 90-100%. A few recent studies suggested that the efficiency of ELISA tests could be increased by recombinant human tTG (htTG) [11, 16, 17].

The aim of our study was to estimate the validity of htTG- and GptTG-based ELISA for diagnosis of CD.

Materials and methods

Patients

Serum samples were obtained from 50 untreated patients (20 boy and 30 girls), aged 1 and 30 girls), aged 1-12 with CD diagnosed according to the criteria established by ESPGHAN, and 38 patients being on gluten free diet (GFD). The controls comprised sera from 50 non-celiac patients (24 boys and 26 girls), aged 1-12 with normal intestinal biopsy. CD and control patients included to the study were without selective IgA immunodeficiency.

Autoantibody measurements

IgA anti-tTG antibodies were determined in patients’ sera by commercial enzyme linked immunosorbent assays (ELISA) using as antigen guinea pig (Gp) tTG (D-TEK s.a., Belgium) or recombinant human (h) tTG (Eurospital S.p.A Italy). The tests were done according manufacture descriptions. The results were expressed as arbitrary units (AU) of standard human serum.

Biopsy specimens

One hundred (100) small intestinal biopsies were performed with the Crosby capsule: 50 were obtained from untreated patients and 50 from controls. The mucosal histology was assessed according to the modified Marsh scale [13]. Five grades of lesions were distinguished:

0 – preinfiltrative type (normal jejunal histology), villous to crypt ratio (v:c) = 3:1
I - infiltrative type, >30 intraepithelial lymphocytes (IEL)/100 enterocytes, v:c = 3:1
II - hyperplastic type, >30 IEL/100 enterocytes, v:c = 2:1, inflammatroy infiltrate in the mucosa
III – destructive type, >30 IEL/100 enterocytes, infiltrate in the mucosa:
   a) v:c = 1:1
   b) v:c = 1:2
   c) total villous atrophy
IV – hypoplastic type (total villous and crypt atrophy).

Statistics

The sensitivity of assays was determined by division the number of untreated celiac patients with positive test by total number of untreated celiac patients, and then multiplication of this result by 100 was done. The specificity was determined by division of the number of controls with negative test by the number of total number of control patients, and this result was multiplied by 100.

Statistical analysis was performed by ANOVA Kruskal – Wallis test to correlate anti-tTG levels between groups of patients and to correlate anti-tTG levels and degrees of mucosal lesion in untreated patients. Wilcoxon test was used to correlate anti-tTG levels in patients before and on the GFD. P<0,05 was considered significant.

Results

Histological evaluation

Out of 50 biopsies of untreated CD patients, 5 were evaluated as grade IIIa (10%), 11 as grade IIIb (22%), 29 as grade IIIc (58%) and 5 as grade IV (10%). All patients in control group had normal morphology of intestinal mucosa.

Anti-hTTG antibodies

In CD group anti-hTTG antibodies were positive in 47 out of 50 patients (sensitivity 94%), and in none of controls (specificity 100%). Values of antibodies were significantly higher in untreated CD patients than those in GFD patients (p<0,05) and than those in the control group (p<0,05) (Fig. 1). We estimated that 32 of 38 patients strictly complied with the GFD. In patients on exact GFD anti-hTTG antibodies decreased significantly when compared with their initial results (Fig. 2). In those 6 patients who did not take the strict diet anti-hTTG antibodies were on the same level. Decreasing values of IgA antibodies was observed as quickly as in the first 3-6 months after GFD was ordered. There was no correlation between the serological results and different degrees of mucosal changes.

The hTTG antibodies were determined by ELISA and expressed in AU. Statistical analysis performed by ANOVA Kruskal-Willis showed significant differences (p<0,05) between all groups.

Anti- Gp-tTG antibodies

In untreated patients the test was positive in 46 of 50 patients (sensitivity 92%) and in 6 of 50 controls (specificity 90%). The amount of Gp-tTG antibodies in untreated group was significantly higher than in patients on GFD (p<0,05) and in the control group (p<0,05) (Fig. 3). Patients who strictly adhered to the diet (n=32) had significantly lower antibody levels as compared with the initial examination (p<0,05) (Fig. 4). A decrease in antibody level, like in rh-tTG test, was observed as quickly as after the first 3-6 months of diet dura-
**Fig. 1.** IgA anti–htTG levels in patients with untreated CD (1), in patients on GFD (2) and in control group (3)

The htTG antibodies were determined by ELISA and expressed in AU. Statistical analysis performed by ANOVA Kruskal-Wallis showed significant differences (p<0.05) between all groups.

**Fig. 2.** IgA anti-htTG antibody levels in patients on strict GFD

Time:
1 : initial diagnosis
2 : 3-9 months GFD
3 : 10-19 months GFD
4 : 20-29 months GFD
5 : 30-39 months GFD
6 : >40 months GFD

**Anti-Gp-tTG antibodies**

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The GpTG antibodies were determined by ELISA and expressed in AU. Statistical analysis performed by ANOVA Kruskal-Wallis showed significant differences (p<0.05) between all groups.

**Fig. 3.** IgA anti–Gp-tTG titers of patients with untreated CD (1), patients on GFD (2) and in control group (3)

**Fig. 4.** IgA anti-GpTG levels in patients on strict GFD
Discussion

The identification of tTG as the major autoantigen in CD has improved our knowledge of the disease pathogenesis and provided a new important tool in the diagnosis of the disease. It has let to development of ELISA-based assays that detect tTG antibodies. However, antigens applied in anti-tTG ELISA kits vary in source and techniques (genetically engineered or tissue extraction), resulting in variations in sensitivity and specificity of anti-tTG antibodies [11]. Originally, commercially available tTG from guinea pig liver was used for determination of anti-tTG antibodies [5, 6]. After cloning and production recombinant hTG, hTG has been employed as antigen in immunoassays [16, 17]. In this study we compared diagnostic value for CD of commercially available GpTG and hTG-based tests. Our results showed that although the sensitivity of both tests was similar (94% for hTG and 92% for Gp-tTG antibodies), the test with hTG antigen is more specific (100% against 90% of Gp-tTG). These findings are in line with other researches. Wong et al. examined 13 commercially available Gp and hTG antibody ELISA kits showing that the use of hTG for detection of IgA antibodies in associated with superior performance, specifically specificity [18]. IgA GpTG gave false-positive results in patients with autoimmune hepatitis or primary biliary cirrhosis due to the presence of hepatic proteins in the commercial tTG obtained from guinea pig liver [3]. It was shown that tTG antibodies had similar as anti-endomysial antibodies sensitivity and specificity in CD diagnosis, and the diagnostic value of both antibodies was greater than that of anti-gliadin antibodies [1, 4]. In this context, it is interesting to note that the use of hTG instead of GpTG can improve the ELISA-based test. However, it is necessary to stress that the tTG antibody estimation cannot replace intestinal biopsy. We did not observed correlation between the amount of both hTG and GpTG antibodies and the degrees of mucosa changes. Our findings confirm that still the determination of histological markers in intestinal biopsy is the gold standard for CD diagnosis [8].

We observed that GFD induced a significant decline of antibody amounts in both tests in the first 3-6 months after the diet was introduced. In patients who did not strictly adhere to the diet such decrease was not found. These results indicate that anti-tTG could be taken under consideration in monitoring of CD treatment.

In conclusion we suggest that because of higher false-negative and false positive rate the Gp-tTG based ELISA should be replaced by more sensitive and more specific htTG-based ELISA.

References