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Tissue transglutaminase expression in duodenal mucosa of patients with celiac disease and of normal subjects

Normal ve gluten enteropatili hastaların duodenal mukozasında doku transglutaminaz ekspresyonu

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Background/aims: *Our aim in the present study was to investigate tissue transglutaminase expression by immunohistochemistry in duodenal mucosa of patients with celiac disease.* ***Methods:*** *A total of twelve patients with celiac disease were examined. The patients had different clinical and histopathologic degrees of severity and responded to gluten withdrawal with clinical improvement. Ten subjects with various unrelated diseases served as controls. Serum endomysium antibodies were measured by an indirect immunofluorescence method using a commercial kit. Duodenal biopsy specimens were stained with hematoxylin and eosin. Immunohistochemical staining for anti-tissue transglutaminase antibodies was performed using commercial kit.* ***Results:*** *Serum endomysium antibodies and evaluation of small bowel biopsy specimens were normal in all control subjects. However, serum endomysium antibodies were positive in all of the celiac patients. Immunohistochemical staining pattern of duodenal biopsy specimen performed using anti-tissue transglutaminase antibodies was similar in celiac patients and control subjects.* ***Conclusion:*** *Tissue transglutaminase expression by immunohistochemical methods in untreated celiac mucosa is not suitable for diagnosis of celiac disease.*

Key words: Celiac disease, anti-tissue transglutaminase antibody, anti-endomysial antibody

Amaç: *Çalışmamızın amacı gluten enteropatili hastaların duodenal mukozasında immünhistokimyasal olarak doku transglutaminaz ekspresyonunu tespit etmektir.* ***Yöntem:*** *Farklı klinik ve histopatolojik özelliklere sahip, anti-endomysium antikoru pozitif ve uygulanan glutensiz diyete yanıt veren gluten enteropatili 12 hasta, değişik nedenlerle üst gastrointestinal sistem endoskopisi yapılan ve duodenal biyopsi örnekleri normal olarak değerlendirilen, anti-endomysium antikorları negatif 10 şahıs kontrol grubu olarak çalışmaya alındı. Serum endomysium antikorları ticari kit kullanılarak immunfloresans yöntemi ile ölçüldü. Tüm duodenal biyopsi örnekleri hematoksilen-eosin ile boyandı Doku transglutaminaz ekspresyonu için ticari kit kullanılarak immünhistokimyasal boyama yapıldı.* ***Bulgular:*** *Serum anti-endomysium antikoru ve duodenum biyopsi incelemeleri tüm kontrol grubunda normaldi. Oysa anti-endomysium antikoru tüm hasta grubunda pozitif bulundu. Doku transglutaminaz antikoru kullanılarak duodenal biyopsilere uygulanan immünhistokimyasal boyama paterni hem kontrol grubunda hem de hasta grubunda benzerdi.* ***Sonuçlar:*** *Tedavi edilmemiş glutenli hastaların duodenal mukozasında immünhistokimyasal olarak saptanan doku transglutaminaz ekspresyonu gluten hastalığının teşhisi için uygun değildir.*

Anahtar kelimeler: Çölyak hastalığı, anti-doku transglutaminaz antikoru, anti-endomysium antikor

INTRODUCTION

Celiac disease (CD) is an autoimmune enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. The ingestion of prolamines (alcohol soluble fractions of wheat, barley, and rye) leads to immunologically mediated, self-perpetuating, small intestinal mucosal damage; the elimination of these substances results in full mucosal recovery (1-4). CD is histologically identified by detection of villous atrophy in small-bowel biopsy specimens. Although biopsy remains the "gold standard" for the diagnosis of CD, serologic testing is valuable as a screening tool (5).

The characteristic IgA autoantibody recognizing the endomysium has been proposed as the most reliable serologic marker for CD (2, 4). These autoantibodies can be identified by an immunofluorescence assay, using either monkey esophagus or human umbilical cord as tissue substrate, with a diagnostic sensitivity of 68-100% and a specificity of 100% when compared with intestinal biopsy (1-5).

Although antibody against endomysium (EMA) measurement is considered to be the best serologic test for CD, the assay does have limitations (5,

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6). The immunofluorescence method is expensive, not suitable for a first-step large-scale screening, and limited by the degree of training of the observer, as it depends on a subjective assessment (2, 5). Another limitation to the use of EMA detection for serologic detection of CD is the increased frequency of IgA deficiency in patients with CD. Thus, sera from CD patients who are also IgA deficient may give a false-negative result for EMA (5).

The discovery by Dieterich et al. (7) that tissue transglutaminase (tTG) is the autoantigen recognized by EMA is seen as a major breakthrough. This enzyme is expressed in many organs and released upon tissue damage (2). It has recently been reported that anti-tTG antibodies has a sensitivity ranging from 86.6% to 98% and a specificity ranging from 90% to 98% in CD (3). However this test has some limitations that would prevent its use as an alternative to the EMA assay (2, 8). In a recent letter, the IgA-tTG ELISA was reported to gave false-positive results in as many as 50% of patients at risk for CD with autoimmune hepatitis or primary biliary cirrhosis, due to the presence of hepatic proteins in the commercial tTG obtained from guinea-pig liver (8).

Our aim in the present study was to investigate the pattern of tTG expression in celiac duodenal mucosa and compare it with normal mucosa, and by that means demonstrate any possible change that may be specific for CD.

MATERIALS AND METHODS

A total of 12 patients with celiac disease (10 female; 2 male; mean age: 25.7 years) were examined. EMA-positive patients had different clinical and histopathologic degrees of severity (malabsorption or partial or subtotal villous atrophy), and responded well to gluten withdrawal. Ten subjects (6 women; 4 men; mean age: 32.6 years) with various unrelated diseases including active chronic gastritis, duodenal ulcer and gastroesophageal reflux disease served as controls.

Endoscopic duodenal biopsy specimens were obtained in all of the patients with celiac disease and in control subjects. The diagnosis of celiac disease was defined with the presence of villous atrophy and crypt hyperplasia.

Serum IgA endomysial antibodies were measured by an indirect immunofluorescence method using a commercial kit (anti-endomysial antibody test system, IMMCO, USA).

Duodenal biopsy specimens were fixed in 10% neutral buffered formalin. Tissues were embedded and cut in paraffin and processed for hematoxylin-eosin staining (H&E). Immunohistochemical staining for anti-tTG antibodies was performed using commercial kit [anti-guinea pig (tTGase), cub 7402, Dako, USA].

RESULTS

The characteristics of patients with CD and controls are shown in (Table 1).

Table 1. The demographic data of patients with celiac disease and controls.

	Controls	**Celiac disease**
Patients (n)	10	12
Gender (F/M)	6/4	10/2
Median age (range), years	32. 6 (24-54)	25.7 (9-47)
Anti-endomysium antibody	(-)	(+)

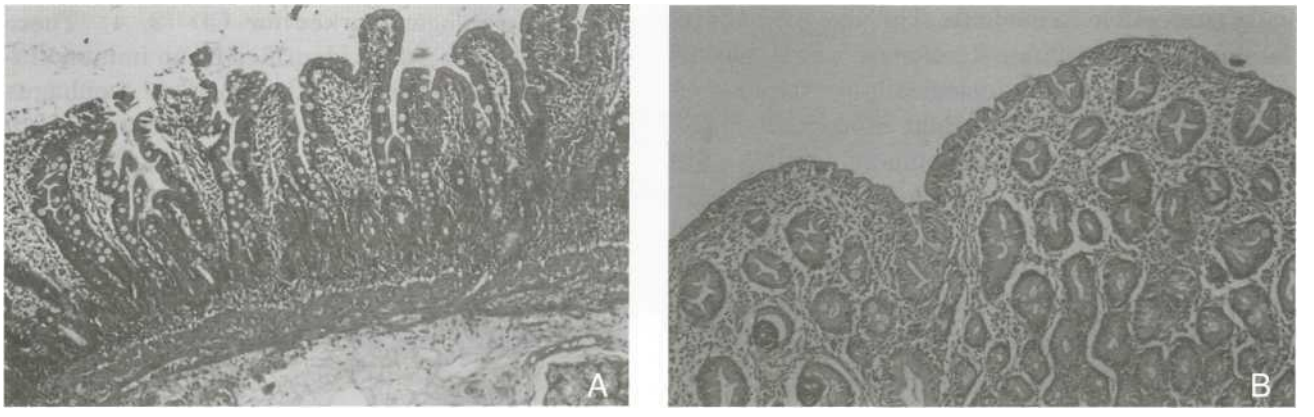

Figure 1. Light micrograph of a section of mucosa obtained by peroral biopsy from the proximal small intestine of a normal subject (A). Duodenal biopsy specimen obtained from a patient with celiac disease (B). The histologic features of severe villous atrophy, crypt hyperplasia, enterocyte disarray and intense inflammatory infiltrate of the lamina propria and epithelial cell layer are evident (H&E, X 25)

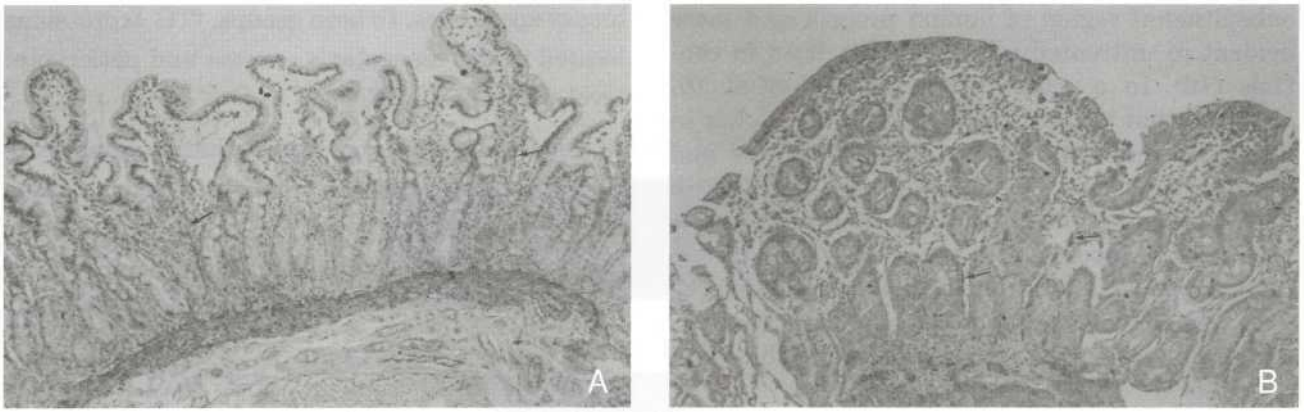

Figure 2. The immunohistochemical staining pattern of duodenal biopsy specimen from healthy subject (A) and celiac patient (B) with anti-tTg antibody. tTG expression (arrows) is seen at muscularis mucosa and pericryptal fibroblasts. The staining pattern is similar in controls and celiac patients (tTG; original magnification, X 25).

Serum EMA and evaluation of small bowel biopsy specimens were normal in all control subjects (Fig. 1A). By contrast, serum EMA were positive in all of the celiac patients. The histological features of duodenal biopsy specimens showed severe villous atrophy, crypt hyperplasia, enterocyte disarray, and intense inflammatory infiltrate of the lamina propria and epithelial cell layer in celiac disease (Fig. IB).

The immunohistochemical staining pattern of duodenal biopsy specimens from celiac patients and healthy subjects with anti-tTG antibody showed a tissue transglutaminase expression at muscularis mucosa and pericryptal fibroblasts. The staining pattern was similar in all controls and celiac patients (Fig. 2).

DISCUSSION

Celiac disease is histologically identified by detection of villous atrophy in small-bowel biopsy specimens. Young patients typically present with failure to thrive, diarrhea and malabsorption; adults, in contrast, may exhibit a vast array of symptoms, including dermatitis herpetiformis, recurrent abdominal pain, and anemia (9,10). Gliadin exposure in CD patients also leads to the production of autoantibodies that recognize endomysium, an intermyofibril substance found in primate smooth-muscle connective tissue (11). Recent studies have identified transglutaminase as the major autoantigenic component of endomysium (7). Although biopsy remains the gold standard for the diagnosis of CD, serologic testing is valuable as a screening tool (5). The single best serologic test for CD is EMA detection, on the basis of its high sensitivity and specificity (>95%) (12). Although EMA measurement is considered to be the best serologic test for CD, the assay does have limitations. In children less than two years old, sensitivity of EMA test is less than 90%. Another limitation to the use of EMA is the increased frequency of IgA deficiency in patients with CD, which may give rise to false-negative results. Approximately 3% of CD patients exhibit IgA deficiency, which has a prevalence of only 0.3% in the general population (5,12). This limitation has led some investigators to recommend that IgA levels be measured in all sera screened for CD by use of EMA (5, 13).

The antigen recognized by IgA EMA has recently been identified as tTG. It has recently been reported that the guinea-pig transglutaminase-based (gp-tTG) enzyme-linked immunosorbent assay used for diagnosis of CD has a sensitivity ranging from 86% to 98% and a specificity ranging from 90% to 98% (3). In a recent letter, the IgA gp-tTG ELISA was reported to gave false-positive results in as many as 50% of patients with autoimmune hepatitis or primary biliary cirrhosis who are at risk for CD. This may be due to the presence of hepatic proteins in the commercial tTG obtained from guinea-pig liver (8).

Tissue transglutaminase is an intracellular enzyme. Biochemically it can be detected in all organs. But it has been shown that the widespread organ distribution of the enzyme is the consequence of its occurrence in ubiquitous cell types such as endothelial and smooth muscle cells (14). Transglutaminase activity is increased in the mucosa of patients with celiac disease (15). Tissue transglutaminase was mainly expressed in the

subepithelial region of lamina propria and more evident in untreated celiac patients than in controls (16). In a recent study, Brusco et al. (6) observed that tTG expression is slightly higher in untreated duodenal mucosa than in treated and normal mucosa. The expression of tTG in pericryptal fibroblasts adjacent to enterocytes is particularly important. In our study, there was no difference in staining patterns between patients with celiac disease and the control group in immunohistochemical examinations of duodenal biopsy specimens. In both groups, tTG expressions located in the muscularis mucosa and pericryptal level were similar (Fig. 2).

In conclusion, our data suggest that immunohistochemical study of tTG expression in celiac mucosa in untreated cases is not a suitable tool for the diagnosis of celiac disease. Further research would be needed to clarify the exact value of the assay.

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