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IgA and IgG Tissue Transglutaminase Antibody Prevalence and Clinical Significance in Connective Tissue Diseases, Inflammatory Bowel Disease, and Primary Biliary Cirrhosis

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An association between celiac disease (CD) and other autoimmune diseases such as connective tissue diseases (CTD), inflammatory bowel diseases (IBD), and primary biliary cirrhosis (PBC) has been reported in several studies. However, a high rate of false positives in autoantibody testing was noted, especially when tissue transglutaminase (tTG) from guinea pig liver was used. Thus, the real prevalence of CD in CTD, IBD, and PBC is unclear. In a case–control study, 400 patients with CTD, 170 with IBD, 48 with PBC, and 120 healthy subjects were investigated for CD by the analysis of IgA and IgG tTG antibodies using the more specific human recombinant tTG immunoenzymatic assay. Patients and controls with positive findings were further tested for antiendomysial antibodies by indirect immunofluorescence and HLA typing, and those found positive by either of these tests underwent duodenal biopsy to confirm a possible diagnosis of CD. Twelve patients were positive for IgA or IgG tTG antibodies, showing an overall prevalence of 1.9%. Only 1 healthy subject (0.8%) had a low level positive reaction for IgA anti-tTG. Among the 12 patients and the healthy subject, only 2 (1 SLE and 1 ulcerative colitis patient) were subsequently confirmed to be affected with CD by positive EMA, HLA, and small bowel biopsy findings. The highest rate of false positives was found in PBC patients (10.4%). For these reasons, serological screening testing for CD is not recommended in CTD patients or in subjects affected with IBD or PBC, unless there is a relevant clinical suspicion of CD.

KEY WORDS: celiac disease; connective tissue diseases; inflammatory bowel diseases; primary biliary cirrhosts; tissue transglutaminase antibodies.

Celiac disease (CD) is a gluten-sensitive enteropathy of autoimmune origin, characterized by inflammation and villous atrophy of the small bowel mucosa, affecting mainly susceptible individuals bearing the HLA DQ2-DQ8 haplotype (1). There is growing evidence that CD is a relatively common disorder, present in approximately 1 in every 180–200 individuals and that most of them are aymptomatic or have only mild gastrointestinal symptoms that may go unrecognized (2–6).

CD was shown to be closely associated with some autoimmune diseases, such as insulindependent diabetes

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mellitus (7, 8), thyroid disorders (9–11), autoimmune myocarditis (12), and Addison's disease (13, 14). Less frequently, CD has also been associated with other autoimmune diseases, including connective tissue diseases (CTD) (15–26) and autoimmune diseases of the digestive tract, namely, inflammatory bowel disease (IBD) (27–30) and primary biliary cirrhosis (PBC) (31–38). However, most of these studies consist of case reports that describe, for the most part, individual cases. Hence, the real prevalence of CD in these autoimmune diseases is still undefined.

The serological diagnosis of CD has been based for years on the detection of IgA and IgG anti-gliadin (AGA) and IgA anti-endomysial antibodies (EMA) (39). Recently, tissue transglutaminase (tTG) has been identified as the main endomysial autoantigen (40), and enzymelinked immunosorbent assays (ELISA) for detecting anti-tTG antibodies have been set up. These new assays were shown to be very sensitive for CD diagnosis; however, a high rate of false positives was reported (41–43), especially when guinea pig liver extracts were used as a source of tTG (42, 44, 45). The recent use of highly purified or recombinant human antigen has improved the specificity of the test, which is now very close to that of EMA (30, 43, 46–50).

In a case–control study, we assessed IgA and IgG anti-tTG antibody prevalence using the more specific human recombinant tTG antigen, in a large series of patients with CTD and autoimmune diseases of the digestive tract, and verify whether findings were associated with clinical features of CD or if a higher rate of false positive results should be expected in these patients.

MATERIALS AND METHODS

The study group comprised 618 adult patients: 100 had systemic lupus erythematosus (SLE), 100 systemic sclerosis, 100 Sjögren's syndrome, 100 rheumatoid arthritis, 48 PBC, and 170 IBD [100 ulcerative colitis (UC), 70 Crohn's disease]. All patients met the American College of Rheumatology or other accepted international criteria for their disease (51–56), and none of them had a previous diagnosis associated with CD. As controls, 120 healthy blood donors were also studied. An oral informed consent was obtained from each patient; patients and controls with positive anti-tTG and EMA results were offered intestinal biopsy to confirm a possible CD diagnosis.

IgA and IgG anti-tTG antibodies were assayed with an ELISA method using an *E. coli*-expressed human recombinant tTG (Eurospital, Trieste, Italy) as the coating antigen. Measurements were done in the same laboratory and by a single operator; sera were thawed only once before determinations. Antibody concentrations were expressed in arbitrary units (AU), as a percentage of one reference calibrator. Cutoff values were set at 7 AU for IgA and at 30 AU for IgG, according to results obtained in a large study previously performed in our institutions (50). All samples with an IgA or IgG value above the cutoff were tested for IgA and IgG EMA by the indirect immunofluorescence method on monkey esophagus sections (Eurospital). If EMA was also positive, the patient underwent intestinal biopsy; if EMA was negative, HLA DQα1 $*$ 0501–DQβ1 $*$ 0201 allele determination was performed (Protrans HLA Celiac Disease, Kesch, Germany), and patients bearing this HLA phenotype, which is strictly associated with CD (57), were subjected to intestinal biopsy.

Table 1. IgA and IgG Anti-tTG Antibody-Positive Results in Autoimmune Patients and Healthy Subjects

		Anti-tTG positive			
		IgA		*IgG*	
*Disease**	*Patients* (N)	N	%	N	%
SLE	100	1	1	2	2
RA	100	1	1	0	0
SSc	100	1	1	0	0
SS	100	0	0	1	1
PBC	48	4†	8.3	4†	8.3
UC	100	1	1	0	0
Crohn's	70	0	0	0	0
Total	618	8	1.3	7	1.1
Healthy subjects	120	1	0.8	0	0

*SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SSc, systemic sclerosis; SS, Sjögren's syndrome; PBC, primary biliary cirrhosis; UC, ulcerative colitis.
†Three PBC patients were positive for both IgA and IgG.

RESULTS

Frequency of IgA and IgG Tissue Transglutaminase Antibodies. Of 618 patients examined, 12 (1.9%) had anti-tTG antibody levels above the cutoff: 8 were positive for IgA, and 7 for IgG (in 3 patients both IgA and IgG were elevated) (Table 1). IgA anti-tTG antibody concentration ranged from 7.1 to 20 AU, and IgG anti-tTG from 33.7 to 60.9 AU, but most results were close to cutoff values (Table 2).

With regard to the type of disease, anti-tTG was positive in 3 SLE patients (1 IgA and 2 IgG), 1 rheumatoid arthritis (IgA), 1 systemic sclerosis (IgA), 1 Sjögren's syndrome (IgG), 5 PBC (10.4%) (1 IgA, 1 IgG, and 3 both IgA and IgG), and 1 UC (IgA). There were no positive results for

Table 2. Antibody Concentration in Anti-tTG-Positive Patients

	Anti-tTG	
	IgA (cutoff 7 AU)	*IgG (cutoff 30 AU)*
SLE	20*	42.3–45.6
RA	8.9	
SSc	7.1	
SS		60.9
PBC	8.1–9.9–8.1–10.4	33.7–41.7–39.9–57
UC	14.3*	
Healthy subject	8.0	

*diagnosed with CD by intestinal biopsy.

IgA or IgG in Crohn's disease patients. Only one of 120 healthy subjects (0.8%) had a low positive reaction (8 AU) for IgA anti-tTG.

Anti-Endomysial Antibodies in Anti-tTG-Positive Patients. IgA EMA were positive in 2 IgA anti-tTG-positive patients (1 SLE and 1 UC); the other 10 anti-tTG-positive patients and the healthy control were all negative for IgA and IgG EMA.

HLA Testing. Of the two EMA-positive patients, one was found positive for the HLA DQα1$*$0501–DQβ1$*$0201 haplotype; the test was not performed in the other patient. Both underwent endoscopic small bowel biopsy to confirm CD diagnosis. The 10 EMA-negative subjects were all found negative, and according to the study protocol, intestinal biopsy was not performed.

Prevalence of Celiac Disease. Both the IgA-tTG-positive EMA-positive patients were found to have histological features of CD as a result of a small-bowel biopsy. The first of these patients was a 24-year-old woman with SLE. She did not complain of gastrointestinal symptoms but had a mild anemia due to iron deficiency. A moderate increase in transaminase levels was occasionally observed. The second patient, a 42-year-old woman with UC, had chronic anemia due to iron deficiency, low body weight, and abdominal distension after meals, all features attributed to her disease. Therefore, a conclusive diagnosis of CD according to ESPGAN criteria (58) was made in 1 of 400 patients with CTD (0.3% prevalence) and in 1 of 170 patients with IBD (0.6%). None of the 48 PBC patients had associated CD.

DISCUSSION

The enzyme transglutaminase, which is synthesized by a broad spectrum of cell types and is widely distributed in human organs, plays an important role in the apoptotic processes because it stabilizes the apoptotic bodies and limits the leakage of intracellular components into the extracellular space (59). The interest in this enzyme has grown since it was seen that tTG was the target protein of anti-endomysial antibodies (40), a diagnostic marker for CD. As a result, immunoenzymatic tests for anti-tTG antibody detection were developed and became commercially available. These new assays are increasingly used in clinical laboratories as a screening test for CD, because of their higher sensitivity in comparison with AGA and EMA (60).

Apart from endocrinological autoimmune disorders, whose association with CD has been established in several studies, CD has been also occasionally associated with CTD, including vasculitis (15, 16), polydermatomyositis (17–19), SLE (16, 20–22), Sjögren's syndrome (16, 23, 24), scleroderma (16), juvenile chronic arthritis (25), and rheumatoid arthritis (16, 23, 26), and with IBD (27–30) and PBC (16, 31–38). However, two main aspects make it difficult to evaluate the actual prevalence of these supposed associations: on one hand, some of these findings are based only on the occurrence of positive antibodies and not on biopsy-proven cases; on the other hand, it is clear how common false positive reactions are in these patients (61). In fact, many of the above studies were performed with tTG extracted by guinea pig liver, which is a definite source of non-specific reactivity (34, 41, 62–64). Indeed, immunoblot with purified guinea pig liver extracts showed that the majority of false positives were due to IgA reactivities to contaminant liver proteins (44, 45).

In evaluating positive anti-tTG finding in patients with an autoimmune disease, four possible situations should be considered:

1. Anti-tTG is a true positive, and CD is associated with the disease. It has been shown that patients affected by a particular CTD, such as Sjögren's syndrome, are more prone to develop CD. Of the 34 Sjögren's syndrome patients studied by Iltanen et al by means of immunological and genetic tests, and small bowel biopsy, 5 (14.7%) were found to have CD, a frequency that is 30–40 times higher than that observed when the population is screened (24), suggesting that the association is not coincidental.

2. Anti-tTG is a true positive, but the patient is not affected by CD. It was suggested that increased antibody levels might result from a non specific low-level IgA or IgG autoantibody production against tTG (46), a condition that might be found in patients with autoimmune diseases characterized by a marked B-polyclonal activation (65, 66). In this case we should be dealing with both a diagnostic false positive and an analytical true positive.

3. Anti-tTG is a true positive, the patient is not affected by CD, but will develop the disease in the following years. CD is highly prevalent in the general population. In most cases, gastrointestinal signs and symptoms are absent; this is the reason why this illness is diagnosed with increasing frequency in adults (67). Recently, very sensitive and specific laboratory tests have been available that allow for the detection of cases of silent CD in which, even in the presence of anti-tTG antibodies in HLA DQ2-DQ8-positive subjects, the damage to the villous architecture or the presence of a phlogistic process is not demonstrable from a histological point of view. These cases will eventually develop clinically evident gluten intolerance (68).

4. Anti-tTG result is a false positive. As mentioned above, false positive results in antibody testing for CD are frequent in autoimmune diseases. One example of this situation is evident from the study of Rensch et al, who

studied 103 SLE subjects, 24 (23.3%) of whom were AGA positive but none was EMA positive and none had intestinal alterations compatible with CD (61).

Thus, the real prevalence of both anti-tTG antibodies and CD in patients with CTD and in patients with autoimmune diseases of the digestive tract is still unclear and needs to be reevaluated. Therefore, we investigated a large number of subjects with these pathologies using the more specific human recombinant tTG assay as a screening test and confirmation of positive results by EMA, genetic HLA determination, and intestinal biopsy. Together with IgA anti-tTG, we also studied IgG anti-tTG. Only very recently, have assays for measurement of IgG anti-tTG become available, and although only a few studies have been performed on this antibody class (30, 60, 66, 69, 70), they have diagnostic value in IgA-deficient patients and may identify a subset of CD subjects without IgA deficiency who are negative for EMA and IgA anti-tTG (30, 60, 70).

We found 12 anti-tTG positive cases in 618 patients, but only two of these were EMA positive and had a positive small bowel biopsy, showing a CD prevalence of only 0.3%, a prevalence rate that is exactly the same as that observed in the general population. Therefore, while our findings demonstrate that anti-tTG antibodies can be detected in about 1–2% of autoimmune patients, for the most part they are false positives.

In contrast with Iltanen's data that emphasized the presence of CD in about 15% of the subjects suffering from Sjögren's syndrome (24), not one of our 100 patients was affected by CD. This discrepancy may be explained by the fact that there could possibly have been differences in the selection of patients, and, above all, by the fact that, while we used a serological screening with biopsy confirmation of only those cases that were proven to be seropositive, Iltanen et al directly performed jejunal biopsy on all their patients. Interestingly enough, of their five patients affected by CD, only three were EMA positive. This points out how a segment of subjects can be affected by CD regardless of the negative results of serological tests; on the other hand, it also possible that EMA-negative subjects with a positive biopsy can be affected by gastrointestinal pathologies different from CD (Iltanen's study does not indicate if, after a gluten-free diet, there was normalization of the histological picture in EMA-negative subjects). Thus, it is possible that our study shows a certain underestimation of CD cases; however, we did not deem it to be ethically appropriate to perform an intestinal biopsy on asymptomatic, anti-tTG and EMA-negative subjects in the absence of a CD-associated HLA-haplotype.

The high rate of false positive results (>10%) we found in PBC patients is not surprising since false positive results have been already reported in PBC. In two independent studies on CD prevalence in autoimmune diseases (44, 45), the highest frequency of false positives was found in patients with liver diseases, with up to 50% testing positive among those with autoimmune hepatitis or PBC. Similar results were obtained by Gillet et al (37), who studied 378 patients with PBC, and found that 10 (2.6%) were positive for IgA guinea pig anti-tTG and EMA antibodies. However, a further 44 patients (11.6%) had raised titers of IgA anti-tTG but were negative for EMA. Since we did not use guinea pig tTG but the more specific human recombinant antigen, this explanation cannot be applied to our findings. One possibility is that IgA and IgG anti-tTG levels may be generally higher in PBC patients than those in the general population or in patients with other autoimmune diseases. Studies are in progress using different tTG sources (ie, red blood cells, human placenta, different human recombinant antigens) to verify the analytical specificity and possible cross-reactivity between anti-tTG and anti-mitochondrial antibodies.

In conclusion, the results of the present study, the largest ever performed using human recombinant tTG assay in patients with CTD or autoimmune diseases of the digestive tract, show that in these diseases, with the exception of PBC, which showed a higher rate of false positive results, tTG antibody and CD prevalences are the same as those observed when the general population is screened. CD is certainly an autoimmune pathology and, as such, it is most likely to be associated with certain organ-specific autoimmune pathologies such as thyroiditis and type 1 diabetes, but not to all autoimmune illnesses. For this reason, serological screening testing for CD is not recommended in CTD patients or in subjects affected with IBD or PBC, unless a clinical suspicion of associated CD is present.

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