

Tissue Transglutaminase Autoantibodies in Patients with IgM Rheumatoid Factors

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The recent identification of tissue transglutaminase (tTG) as the autoantigen for celiac disease-associated anti-endomysial antibodies (EMA) has allowed the use of rapid immunoassay to detect the presence of autoantibodies, anti-tTG, in the serum of patients. In this study, we examined the prevalence of IgG or IgA anti-tTG in sera from patients with elevated levels of IgM rheumatoid factors, which are autoantibodies reactive with the Fc portion of IgG. We report here on four cases of anti-tTG positivity for patients with elevated IgM rheumatoid factor (RF) without evidence of celiac sprue.

The study population consisted of 65 patients (26 men, 39 women; mean age, 49 years; range 4 - 92 years) with elevated RF (>20 U/ml), and 23 healthy subjects (12 men, 11 women; mean age, 46 years; range, 21 - 54 years). IgG and IgA anti-tTG levels were detected using a commercially available ELISA kit (Immuno-Biological Laboratories, Germany).

Out of 65 patients, one (1.5%) and three (4.6%) patients were positive for IgG and IgA anti-tTG antibodies, respectively, and this was a higher frequency than occurred in healthy subjects (0/23). The clinical features of the four cases positive for IgG or IgA anti-tTG were as follows:

The first case (female, 63 yrs) positive for IgA anti-tTG antibody suffered from rheumatoid arthritis, type II diabetes mellitus, iron deficiency anemia and gastric indigestion without symptoms of malabsorption. She denied any gluten sensitivity on her diet. Her esophagogastroduodenoscopic biopsy showed mucosal atrophy with no elongated crypts or infiltration of inflammatory cells in the lamina propria. The remaining three cases positive for anti-tTG antibodies had interstitial pneumonia, a herniated lumbar disc, and mild scoliosis, respectively. They all denied any malabsorption symptoms or gluten

sensitivity. Jejunal biopsy could not be performed in all four cases.

Key Words: Anti-tissue transglutaminase antibodies, autoimmunity, rheumatoid factor

The etiology of celiac sprue is not known, but environmental, genetic and immunological factors all appear to contribute to the disease.¹ An important and complex role for the enzyme tissue transglutaminase (tTG) and autoimmunity have been suggested in the pathogenesis of celiac disease.² The recent identification of tTG as the autoantigen for celiac disease-associated anti-endomysial antibodies (EMA) has allowed the use of rapid immunoassay to detect the presence of autoantibodies, anti-tTG, in the serum of patients.^{3,4} Many studies on the clinical application of anti-tTG determination have reported a very high sensitivity and specificity for the diagnosis of celiac disease.⁵⁻⁸ However, anti-tTG can be found in other gastrointestinal⁹ or autoimmune diseases.¹⁰ In this study, we examined the prevalence of IgG or IgA anti-tTG in the sera from patients with elevated levels of IgM rheumatoid factors, which are autoantibodies reactive with the Fc portion of IgG. As a result, here we report on four cases of anti-tTG positivity for patients with elevated IgM rheumatoid factor (RF) without evidence of celiac sprue.

The study population consisted of 65 patients (26 men, 39 women; mean age, 49 years; range 4-92 years) with elevated RF (>20 U/ml), and 23 healthy subjects (12 men, 11 women; mean age, 46 years; range 21-54 years). The patient group included 16 with autoimmune disease (13 with rheumatoid arthritis, 1 each with periarteritis

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nodosa, Sjogren syndrome and Graves's disease), 28 with neuromuscular disease (2 with osteoarthritis, 7 with radiculopathy, 12 with herniated disc, 4 with spondylitis, 1 each with transverse myelitis, progressive muscular dystrophy and scoliosis), 8 had febrile or inflammatory disease (3 with hepatitis, 2 with pneumonia, 1 each with meningitis, cholecystitis and *Epstein-Barr virus* infection,) and 13 other diseases (2 with stroke, 3 with anemia, 3 with chronic superficial gastritis and 3 with renal failure, 1 each with congestive heart failure and lymphoma). IgM rheumatoid factor was measured by the nephelometric method (Dade Behring, Marburg, Germany). IgG and IgA anti-tTG levels were measured using a commercially available ELISA kit (Immuno-Biological Laboratories, Germany). The intra-assay coefficient of variation assessed by a ten-fold determination in positive serum samples was less than 10%. We defined those anti-tTG values higher than 12 U/mL as positive according to the manufacture's indication. Frequency analysis was done by McNemar test and the comparison of antibody levels between the patient and normal groups was tested with a Mann-Whitney U test. A Pearson coefficient was used for correlation between RF and anti-tTG levels. P values of less than 0.05 were regarded as significant.

The distribution of antibody levels in patients and healthy subjects is summarized in Fig. 1. Out of 65 patients, one patient (1.5%) and three others (4.6%) were positive for IgG and IgA anti-tTG antibodies, respectively, and this was a higher frequency than occurred in healthy subjects (0/23) ($p < .0001$). The clinical features of the four cases positive for IgG or IgA anti-tTG are summarized in Table 1. The first case (female, 63 yrs) positive

for IgA anti-tTG antibody suffered from rheumatoid arthritis, type II diabetes mellitus, iron deficiency anemia, and gastric indigestion without symptoms of malabsorption. She denied any gluten sensitivity in her diet. Her esophagogastroduodenoscopic biopsy showed mucosal atrophy without elongated crypts or infiltration of inflammatory cells in the lamina propria. Jejunal biopsy could not be performed due to the patient's refusal. The remaining three cases positive for anti-tTG antibodies had interstitial pneumonia, a herniated lumbar disc and mild scoliosis, respectively. They all denied any symptoms of malabsorption or gluten sensitivity. Jejunal biopsy could not be performed in all three cases. There was no correlation between IgM RF and the IgG or IgA anti-tTG antibody levels. However, there is a significant correlation between IgG and IgA anti-tTG antibody levels in samples studied ($n=88$, $r=$

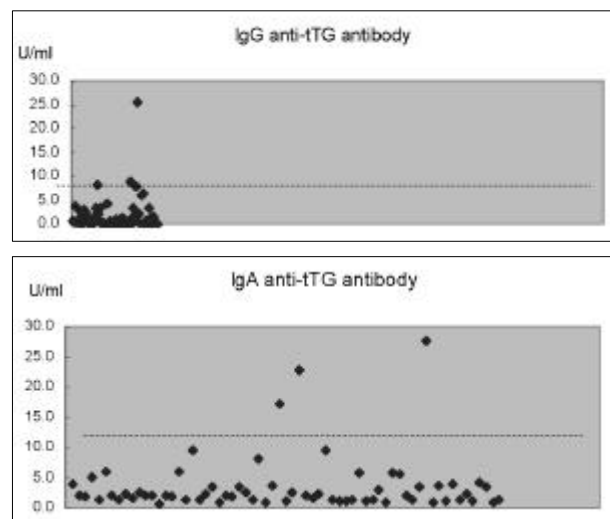


Fig. 1. Serum IgG and IgA anti-tTG antibodies in patients with elevated RF levels.

Table 1. Clinical Features of Four Cases Positive for IgG or IgA Anti-tTG Antibodies (U/mL)

Case No.	Sex	Age (year)	IgG anti-tTG	IgA anti-tTG	GI Sx	RF (IU/mL)	CRP [†]	Clinial diagnosis
1	f	63	-	27.7	+	26.9	+	RA, DM
2	f	44	25.4	-	-	179	+	pneumonia
3	f	39	-	22.8	-	1230	-	HLD
4	f	16	-	17.2	-	25.4	-	scoliosis

tTG, tissue transglutaminase; GI Sx, gastrointestinal symptom; RF, rheumatoid factor; CRP, C-reactive protein; RA, rheumatoid arthritis; DM, diabetes mellitus (type 2); HLD, herniated lumbar disc.

*gastric indigestion with no malabsorption.

[†] cut-off (4 mg/L); 68 mg/L in case No 1, 36.3 mg/L in case No 2.

0.2501, $p=0.0187$).

The advantage of using tTG testing is that ELISA eliminates the disadvantages associated with the use of EMA, namely the time-consuming protocol, difficulty in standardization, and subjective interpretation of the immunofluorescence assay. Reviewing the results of recent studies using the human tTG as a substrate, anti-tTG antibody test compares well with EMA for sensitivity,¹¹ and it can be used as an alternative to the EMA assay.¹² However, there is a loss of specificity, especially for patients with autoimmune diseases.¹³ According to a recent study,¹⁴ 11% of samples from patients with rheumatoid arthritis were positive for anti-tTG antibodies. In this study, 4 (6.1%) of 65 serum samples were positive for IgG or IgA anti-tTG. All four cases positive for anti-tTG were unlikely to have celiac disease. The limitation of this study was that confirmatory intestinal biopsy could not be performed. Because celiac disease often goes undiagnosed and the clinical manifestations may be atypical, further large prospective studies are needed. However, in a previous study on antinuclear antibodies, high concentrations of IgG anti-tTG were found for patients with systemic lupus erythematosus. This suggests there is the link between apoptosis and autoimmunity.¹⁵ In conclusion, we suppose that presence of anti-tTG antibodies may be an apoptosis-associated event in inflammatory diseases.² In addition, given that tTG antigen is expressed in many organs and it is released upon tissue damage, we assume that the limited specificity of anti-tTG antibody assay is due to the occurrence of autoantibodies directed against an antigen different from tTG as an immune response for various clinical conditions. However, the clinical significance of positivity for anti-tTG antibodies in relationship with celiac disease should also be investigated for those patients with autoimmune disease suffering from gastrointestinal symptoms.

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