Comparison of IgA endomysium antibody and IgA tissue transglutaminase antibody in celiac disease

Helen R Gillett MD, Hugh J Freeman MD

HR Gillett, HJ Freeman. Comparison of IgA endomysium antibody and IgA antibody to tissue transglutaminase in celiac disease. Can J Gastroenterol 2000;14(8):668-671. The antigen for immunoglobulin (Ig) A endomysium antibody (EmA), a sensitive and specific serological marker for celiac disease, has recently been described as tissue transglutaminase (tTG). The aim of this study was to compare the assays used to measure IgA EmA and IgA tTG antibody in patients with celiac disease and disease control subjects. Sera from 21 patients with untreated celiac disease, 48 patients with treated celiac disease and 128 disease control subjects were tested both for IgA EmA with the use of indirect immunofluorescence against human umbilical cord and for IgA tTG antibody with the use of ELISA.

Titres of IgA tTG antibody were significantly higher in both the untreated and treated celiac groups than in the disease control group. Titres in the treated group were, however, significantly lower than in the untreated group. A reference range was calculated to include 99.8% of the disease control group in whom small bowel biopsy showed no evidence of celiac disease. One patient from the disease control group with raised IgA tTG antibody titres and positive IgA EmA was found to have celiac disease on small bowel biopsy. The sensitivity, specificity, and positive and negative predictive values of the IgA EmA assay were all 100%. The sensitivity of the IgA tTG antibody assay was 95%, specificity 100%, positive predictive value 100% and negative predictive value 97.7%.

An ELISA used to measure IgA tTG antibody is an excellent tool to screen for celiac disease and may prove useful for monitoring response to treatment.

Key Words: *Celiac disease; Endomysium antibody; Tissue transglutaminase*

Comparaisons entre l'anticorps anti-endomysium IgA et l'anticorps anti-transglutaminase tissulaire IgA dans la maladie coeliaque

RÉSUMÉ : L'antigène de l'anticorps anti-endomysium (EmA) IgA (immunoglobuline A), un marqueur sérologique sensible et spécifique de la maladie coeliaque, a été décrit comme une transglutaminase tissulaire (tTG). L'étude a pour but de comparer le dosage des anticorps EmA IgA et des anticorps tTG IgA chez des patients atteints de la maladie coeliaque et chez des témoins. On a analysé le sérum de 21 patients souffrant de maladie coeliaque non traitée, de 48 patients souffrant de maladie coeliaque traitée et de 128 témoins, à la recherche d'anticorps EmA IgA au moyen de l'immunofluorescence indirecte sur des coupes de cordon ombilical humain et d'anticorps tTG IgA à l'aide du test ELISA.

Le dosage des anticorps tTG IgA dans les deux groupes de sujets atteints de la maladie coeliaque, traitée ou non, était de beaucoup supérieur à celui dans le groupe de sujets témoins, mais celui dans le groupe de sujets traités était passablement inférieur à celui dans le groupe de sujets non traités. On a calculé une plage de référence comprenant 99,8 % des sujets du groupe témoin chez qui une petite biopsie de l'intestin grêle n'a montré aucun signe de maladie coeliaque. Un seul patient dans le groupe témoin s'est avéré porteur de la maladie coeliaque à la biopsie; d'ailleurs, son dosage d'anticorps tTG IgA était élevé et la recherche d'anticorps EmA IgA s'est révélée positive. La sensibilité, la spécificité et les valeurs prédictives positive et négative du dosage des anticorps EmA IgA ont été justes dans 100 % des cas. Quant au dosage des anticorps tTG IgA, sa sensibilité était de 95 %; sa spécificité, de 100 %; sa valeur prédictive positive, de 100 % et sa valeur prédictive négative, de 97,7 %.

La mesure des anticorps tTG IgA à l'aide du test ELISA constitue un excellent outil de dépistage de la maladie coeliaque et peut s'avérer utile pour surveiller la réaction au traitement.

Division of Gastroenterology, Department of Medicine, University of British Columbia, Vancouver, British Columbia
Correspondence and reprints: Dr Helen R Gillett, Gastroenterology – Room F 137, University Hospital, 2211 Wesbrook Mall, Vancouver, British Columbia V6T 1Z3. Telephone 604-822-7216, fax 604-822-7236, e-mail pgillett@interchange.ubc.ca
Received for publication March 19, 1999. Accepted November 30, 1999

Immunoglobulin (Ig) A endomysium antibody (EmA) has been found to be highly sensitive and specific for celiac disease (1-3). EmA is usually detected with the use of indirect immunofluorescence against either monkey esophagus or human umbilical cord, making it, at best, only semiquantitative. In general, therefore, IgA EmA is of little use in monitoring response to a gluten-free diet. The identification of the antigen for endomysium as tissue transglutaminase (tTG) (4) has, therefore, opened up the possibility of using an ELISA to detect and quantify tTG, potentially improving our understanding of its role in the pathogenesis of celiac disease.

We have established an ELISA to measure IgA antibody to tTG and have tested a group of patients with untreated celiac disease, a group of patients with treated celiac disease and a disease control group for this antibody and for IgA EmA with the use of human umbilical cord to compare the sensitivities and specificities of the two tests.

PATIENTS AND METHODS

Sera from 21 patients with untreated celiac disease and 128 disease control subjects were tested for IgA EmA and IgA tTG antibodies. The diagnoses of the disease control group are listed in Table 1. In addition, 54 samples from 48 patients with treated celiac disease were tested. Celiac disease was defined by biopsy in all patients (ie, severe flat lesion responding to a gluten-free diet), and biopsy results of 42 disease control subjects were normal. The other 86 control subjects did not undergo small intestinal biopsies. The control group was, therefore, subdivided into 'biopsy' and 'nonbiopsy' control groups.

IgA EmA was detected with the use of indirect immunofluorescence against human umbilical cord according to the method described by Ladinser et al (2); the serum was measured at a 1:5 dilution. Titres of IgA antibody to tTG were measured with the use of an ELISA method devised by Dieterich et al (5). This technique was modified to account for differences in scientific supplies. In brief, high affinity 96-well microtitre plates (Costar Corporation, USA) were coated with 1/600 U/well of tTG from guinea pig (Sigma-Aldrich Canada Ltd, Canada) in 100 µL 50 mM tris-hydrochloride, 150 mM sodium chloride and 5 mM calcium chloride (pH 7.5) overnight at 4°C. After washing three times with 50 mM tris-hydrochloride, 150 mM sodium chloride, 10 mM EDTA and 0.1% Tween 20 (pH 7.4), the plate was blocked for 10 min at 37°C using this solution. Samples were then applied to the plates at 1:5 dilution using the same solution as a diluent. The plate was incubated at room temperature for 1 h before being washed three times again. Peroxidase-conjugated rabbit antihuman IgA (Dako Corporation, USA) was applied at 1:3000 dilution for 1 h at room temperature, and the plate was again washed three times. O-phenylenediamine-hydrochloride 0.4 mg/mL in 0.05 mM citric acid and 0.1 mM dibasic sodium phosphate with 0.06% hydrogen peroxide was applied to the plate and incubated for 1 h in the dark at room temperature before reading using 450 nm wavelength light. A positive sample was tested in doubling dilutions until the linear portion of the standard curve was obtained. This sample was designated to have 400 arbitrary units (AU)/mL and was used as for the standard curve for each plate. The titre, in AU, of each sample was then calculated from this curve.

Statistical differences were calculated using the Mann-Whitney Test.

TABLE 1
Diagnoses of disease control group

Diagnosis	Number of Patients
Ulcerative colitis	50
Crohn's disease	49
Collagenous colitis	9
Lymphocytic colitis	7
Irritable bowel syndrome	4
Unclassified colitis	3
Cystic fibrosis	1
Colitis cystica profunda	1
Gallstone disease	1
Hemorrhoids	1
Hepatic steatosis	1
Pseudomembranous colitis	1

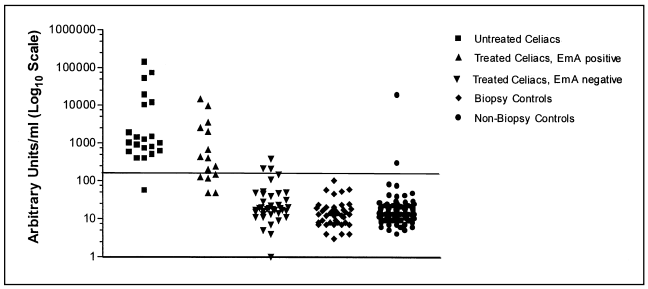

Figure 1) *Titres of immunoglobulin (Ig) A antibody to tissue transglutaminase in patients with untreated celiac disease, patients with treated celiac disease (IgA endomysium antibody [EmA] -positive and -negative), biopsy control subjects and nonbiopsy control subjects. Horizontal lines indicate a reference range of 1.4 to 138.4 arbitrary units/mL*

RESULTS

IgA EmA: All patients with untreated celiac disease were found to be positive for IgA EmA. One patient from the nonbiopsy control group tested positive for the antibody. All patients in the biopsy control group were negative for IgA EmA. Thirty-nine samples from patients with treated celiac disease were negative for IgA EmA, and 15 were positive.

IgA antibody to tTG: The titres of IgA tTG antibody are shown in Figure 1 and Table 2. The titres were significantly higher in the untreated celiac group than in the EmA-negative groups ($P<0.0001$) and the EmA-positive, treated group ($P=0.027$). Titres in the EmA-positive, treated group were significantly higher than in all the EmA-negative groups ($P<0.0001$). The EmA-negative, treated group had higher titres than both the biopsy and the nonbiopsy control groups ($P=0.0072$ and $P=0.0099$, respectively). There was no

TABLE 2
Titres of immunoglobulin A tissue transglutaminase antibodies within each group

Group	Number within group	Range (AU/mL)	Median
Untreated celiac disease	22	55-142,000	1022.0
Treated celiac disease (EmA positive)	15	47-14,500	398.0
Treated celiac disease (EmA negative)	39	<1-386	19.0
Controls (biopsied)	42	3-102	13.0
Controls (not biopsied)	85	4-19,000	13.5

AU Arbitrary unit; EmA Endomysium antibody

TABLE 3
Serial titres of immunoglobulin (Ig) A tissue transglutaminase (tTG) antibodies in six patients with treated celiac disease and four patients with untreated celiac disease

Patient	Time interval (months)	IgA tTG antibody titres (AU/mL)
Treated celiac disease		
1	10, 24	3500, 2500, 14500
2	12	31, 47
3	18	39, 7
4	24	124, 147
5	24	23, 28
6	24	47, 200
Untreated celiac disease		
1	5	73000, 2000
2	7	142000, 9500
3	14	400, 115
4	18	740, 430

AU Arbitrary unit

statistically significant difference between the two control groups.

A reference range for IgA tTG antibody was calculated using the biopsy control group. The titres within this group were converted to $\log_{10}$ to normalize the data. The mean ± 3.1 standard deviations were calculated and antilog_{10} determined. The calculated reference range was 1.4 to 138.4 AU/mL.

Titres of IgA antibody to tTG in patients with treated celiac disease: In the patients with treated celiac disease, seven of 15 (46.7%) samples positive for IgA EmA had IgA tTG antibody titres within the calculated reference range. Of the treated patients who were negative for IgA EmA, 35 of 39 samples (89.7%) had titres within the reference range; three of these samples had titres above 138.4 AU/mL, and one had a titre of less than 1 AU/mL. This patient was found to have selective IgA deficiency (SIgAD).

In five patients, two samples taken 18 to 24 months apart were tested. Three samples were tested from a sixth patient over a two-year period. Three patients were consistently positive for IgA EmA, and three were consistently negative.

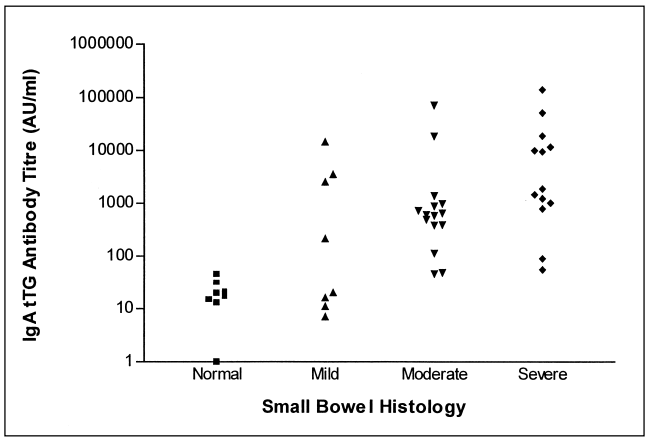

Figure 2) *Titres of immunoglobulin (Ig) A tissue transglutaminase (tTG) antibody in patients with treated celiac disease and patients with untreated celiac disease by severity of small bowel mucosal lesion. Titres from those with normal mucosae differed from titres from those with moderate (P=0.0001) and severe histology (P=0.0002). Titres were higher in those with severe lesions than in those with moderate lesions (P=0.0282). AU Arbitrary Units*

The titres of IgA tTG antibody in these samples are shown in Table 3.

Four patients from the group with untreated celiac disease had samples taken five to 18 months after commencing a gluten-free diet. All remained positive for IgA EmA, but titres of IgA tTG antibody fell in each patient. The titres are also shown in Table 3.

Patients from the control groups with positive antibody results: The one patient from the nonbiopsy control group found to be positive for IgA EmA had an IgA tTG antibody titre of 19,000 AU/mL. He was an elderly man who had been diagnosed many years earlier with ulcerative colitis. He underwent small intestinal biopsy, which demonstrated subtotal villous atrophy and crypt hyperplasia. He, therefore, commenced a gluten-free diet.

No patient from the biopsy control group had elevated titres of IgA tTG antibody. One patient in the nonbiopsy control group – a man with Crohn's disease and a titre of 297 AU/mL – had a titre above the cutoff level. He had a second serum sample tested 10 months later, and the titre had fallen to 17 AU/mL.

IgA tTG antibody titres compared with small bowel histology: Biopsies on all 22 patients with untreated celiac disease (21 diagnosed at the start of the study and one diagnosed as a result of the study) and on 26 patients with treated celiac disease were graded as normal, mild, moderate and severe. Titres of IgA tTG antibody in each of these histological grades are shown in Figure 2.

Titres from those with normal mucosae differed from those with moderate (P=0.0001) and severe lesions (P=0.0002). Titres were higher in those with severe lesions than in those with moderate lesions (P=0.0282).

Sensitivities, specificities, and positive and negative predictive values: All 21 patients in the group with untreated celiac disease plus one patient with confirmed celiac disease

from the nonbiopsy control group were positive for IgA EmA. Because the entire biopsy control group was negative for this antibody, the sensitivity, specificity, and positive and negative predictive values of the assay were all 100%.

One patient with untreated celiac disease had a titre of IgA tTG antibody below 138.4 AU/mL, making the sensitivity of this assay 95.5%. Because everyone in the biopsy control group had titres within the reference range, the specificity was 100%. The positive predictive value, therefore, was 100%, and the negative predictive value 97.7%.

DISCUSSION

Our study shows the excellent correlation between IgA EmA with the use of human umbilical cord and IgA antibody to tTG. Generally, we found markedly elevated titres in the group with untreated celiac disease and lower titres in the treated group. In the treated group, 28% of the patients continued to have elevated titres despite being on a gluten-free diet. The majority of these patients (80%) were also positive for IgA EmA.

The sensitivity and specificity of our IgA tTG assay were comparable with those of the IgA EmA assay using monkey esophagus (2,6,7). Studies using human umbilical cord as the substrate have generally quoted better results (1-3), and this is thought to be a result of using human tissue to detect human antibody. The sensitivity of the tTG antibody assay has also been reported to be improved by using cloned human tTG rather than guinea pig tTG (8), but this is not yet widely available.

IgA EmA has been used to monitor the response to treatment but can take 12 months to disappear (9), making their use in this clinical setting limited. Nevertheless, the presence of EmA despite treatment can signify continued disease activity. We found that the titres of tTG antibody were higher in the treated group positive for EmA than in the equivalent group negative for EmA, suggesting that serial testing of tTG antibody titres would be useful for noninvasive monitoring of the response to treatment. This is also suggested by our finding that higher titres are generally found in more severe histological lesions.

SIgAD is estimated to occur in one in 400 to one in 700 individuals (10); however, the prevalence of SIgAD in patients with celiac disease has been reported to be as high as 2% to 3% (11-13). Relying solely on IgA tissue antibodies such as EmA to detect celiac disease, therefore, would lead to missed diagnosis in these patients. The one patient in our study with SIgAD had a very low titre of tTG antibody (less than 1 AU/mL) – below our calculated reference range of 1.4 to 138.4 AU/mL. The use of IgA tTG antibody with the addition of total IgA levels in patients with very low titres would, therefore, minimize the risks of failure to detect a patient with both SIgAD and celiac disease. The use of the IgG class of tTG antibody may also be useful in detecting such patients.

Serological testing does not replace the need for small intestinal biopsy in the diagnosis of celiac disease, and these tests are aimed at screening for the disease in high risk groups or in patients in whom the index of suspicion of the disease is low. The very high negative predictive value indicates that tTG antibody will make an excellent screening tool because only a small number of patients will undergo small intestinal biopsy for a false positive result. Our one false negative result does, however, reinforce the need for small intestinal biopsy in all patients with symptoms suggestive of celiac disease.

ACKNOWLEDGEMENTS: Research funding for this study was provided to Dr Freeman from the Rhodes Foundation, Vancouver, British Columbia, and the Canadian Celiac Association, Vancouver Chapter.

REFERENCES

1. Volta U, Molinaro N, De Franceschi L, Fratangelo D, Bianchi FB. IgA anti-endomysial antibodies on human umbilical cord tissue for celiac disease screening. Save both money and monkeys. Dig Dis Sci 1995;40:1902-5.
2. Ladinser B, Rossipal E, Pittschieler K. Endomysium antibodies in coeliac disease: An improved method. Gut 1994;35:776-8.
3. Sategna-Guidetti C, Grosso SB, Bruno M, Grosso S. Is human umbilical cord the most suitable substrate for the detection of endomysium antibodies in the screening and follow-up of coeliac disease? Eur J Gastroenterol Hepatol 1997;9:657-60.
4. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797-801.
5. Dieterich W, Laag E, Schöpper H, et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease. Gastroenterology 1998;115:1317-21.
6. McMillan SA, Haughton DJ, Biggart JD, Edgar JD, Porter KG, McNeill TA. Predictive value for coeliac disease of antibodies to gliadin, endomysium, and jejunum in patients attending for jejunal biopsy. Br Med J 1991;303:1163-5.
7. Valdimarsson T, Franzen L, Grodzinsky E, Skogh T, Strom M. Is small bowel biopsy necessary in adults with suspected celiac disease and IgA antiendomysium antibodies? 100% positive predictive value for celiac disease in adults. Dig Dis Sci 1996;41:83-7.
8. Lampasona V, Bazzingaluppi E, Barera G, Bonifacio E. Tissue transglutaminase and combined screening for coeliac disease and type 1 diabetes-associated autoantibodies. Lancet 1998;352:1192-3.
9. Kapuscinska A, Zalewski T, Chorzelski TP, et al. Disease specificity and dynamics of changes in IgA class anti-endomysial antibodies in celiac disease. J Pediatr Gastroenterol Nutr 1987;6:529-34.
10. Koisinen J. Selective IgA deficiency in blood donors. Vox Sang 1975;29:192-202.
11. Collin P, Mäki M, Keyriläinen O, Hällström O, Reunala T, Pasternak A. Selective IgA deficiency and coeliac disease. Scand J Gastroenterol 1992;27:367-71.
12. Cataldo F, Ventura A, Bottaro G, Corazza GR, Italian Society of Paediatric Gastroenterology and Hepatology (SIGEP), "Club del Tenue" Working Groups on Coeliac Disease. Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. Gut 1998;42:362-5.
13. Cataldo F, Marino V, Bottaro G, Greco P, Ventura A. Celiac disease and selective immunoglobulin A deficiency. J Pediatr 1997;131:306-8.