

IgG₁ antiendomysium and IgG antitissue transglutaminase (anti-tTG) antibodies in coeliac patients with selective IgA deficiency

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Abstract

Background—In selective IgA deficiency (IgAD), there is no reliable screening test for coeliac disease (CD).

Aim—To evaluate the usefulness of IgG₁ antiendomysium and IgG antitissue transglutaminase tests for CD diagnosis in IgAD.

Methods—IgA and IgG anti gliadin antibodies (IgA- and IgG-AGA), IgA and IgG₁ antiendomysium antibodies (IgA- and IgG₁-EMA), and IgA and IgG antitissue transglutaminase (IgA- and IgG-anti-tTG) were assayed in: (a) 20 untreated IgAD/CD patients; (b) 34 IgAD/CD patients on a strict gluten free diet (GFD); (c) 10 IgAD/CD patients not on a strict GFD; (d) 11 untreated CD patients without IgAD; (e) 10 healthy IgAD patients; and (f) 25 healthy controls.

Results—In all untreated IgAD/CD patients, IgG₁-EMA, IgG-anti-tTG, and IgG-AGA were positive whereas IgA antibodies against these antigens were negative. IgAD/CD patients on a strict GFD did not produce IgG-AGA or IgG₁-EMA but four of 34 produced IgG anti-tTG. IgAD/CD subjects not on a strict GFD produced IgG-AGA whereas 5/10 and 4/10 were IgG₁-EMA and IgG-anti-tTG negative, respectively. Untreated CD patients without IgAD were AGA (IgA and IgG), EMA (IgA and IgG₁), and anti-tTG (IgA and IgG) positive. Healthy controls were AGA and EMA negative whereas two of 10 apparently healthy IgAD subjects and one of 25 healthy negative control were IgG-anti-tTG positive.

Conclusions—Both IgG₁-EMA and IgG-anti-tTG tests appear to be useful for identification of IgAD/CD patients whereas they are less satisfactory for monitoring dietary compliance in these subjects. In addition, our findings seem to suggest that IgG-EMA autoantibodies produced by coeliac patients are mainly of the IgG₁ subtype.

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Keywords: IgG₁ antiendomysium antibodies; IgG antitissue transglutaminase antibodies; selective IgA deficiency; coeliac disease

Selective IgA deficiency (IgAD) is frequently associated with coeliac disease (CD), with an incidence 10–15-fold higher than in the general

population.^{1–3} For this reason, subjects with IgAD are at risk of CD, and screening for gluten intolerance is mandatory.

In patients with IgAD/CD on a free (gluten containing) diet, IgA anti gliadin antibodies (IgA-AGA), IgA antiendomysium antibodies (IgA-EMA), and the more recently identified IgA antitissue transglutaminase antibodies (IgA-anti-tTG) are not detectable,⁴ whereas only IgG antibodies (for example, IgG-AGA) can be detected. In a previous multicentre study,³ we observed that IgG-AGA were sometimes absent in sera from untreated patients with IgAD/CD; hence in these subjects the usefulness of other diagnostics test for serological screening of gluten intolerance needs to be evaluated.

Recently, other tests (IgG-EMA⁵ as well as IgG-anti-tTG,^{6,7} the main autoantigen recognised by antiendomysial antibodies⁷) have been established. In CD patients, IgA-anti-tTG are generally considered in the diagnosis and follow up of the disease whereas IgG antibodies (both EMA and anti-tTG) seem to be less effective for these purposes and results of IgG antibody tests should be considered in combination with other clinical and laboratory findings.^{8–10}

In this collaborative study we have used a new test that identifies the IgG₁ subclass of EMA (IgG₁-EMA)¹¹ together with immunoenzymatic measurement of IgG anti-tTG autoantibodies (IgG-anti-tTG) to evaluate the usefulness of these tests in the diagnosis and follow up of 64 patients with IgAD/CD. This study was carried out under the auspices of the Italian Society of Gastroenterology and Hepatology (SIGEP) and the “Club del Tenue”.

Patients and methods

PATIENTS

This was a cross sectional nationwide study involving 19 adult and paediatric gastroenterology centres. We performed serum immunoglobulin (IgA, IgG, IgM) assays and coeliac disease related antibody patterns in the following groups of subjects: (1) 20 untreated coeliac patients with IgAD; (2) 34 coeliac patients with IgAD on a strict gluten free diet (GFD) for

Abbreviations used in this paper: Ig, immunoglobulin; IgAD, IgA deficiency; CD, coeliac disease; AGA, anti gliadin antibodies; EMA, antiendomysium antibodies; tTG, tissue transglutaminase; GFD, gluten free diet; PBS, phosphate buffered saline; HSA, human serum albumin; ABS, absorbance.

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Table 1 Subjects positive for antigliadin antibodies (IgG-AGA+; IgA-AGA+), antiendomysium antibodies (IgG-EMA+; IgA-EMA+), and antitissue transglutaminase antibodies (IgG-anti-tTG+; IgA-anti-tTG+) among patients with coeliac disease (CD) affected or not by selective IgA deficiency (IgAD) and in healthy controls

	IgG ₁ -EMA+	IgA-EMA+	IgG-anti-tTG+	IgA-anti-tTG+	IgG-AGA+	IgA-AGA+
Untreated CD patients with IgAD	20/20	0/20	20/20	0/20	20/20	0/20
CD patients with IgAD on a strict GFD	0/34	0/34	4/34	0/34	0/34	0/34
CD patients with IgAD not on a strict GFD	5/10*	0/10	6/10†	0/10	10/10	0/10
Untreated CD patients without IgAD	11/11	11/11	11/11	11/11	11/11	11/11
Healthy IgAD subjects on a gluten containing diet	0/10	0/10	2/10	0/10	0/10	0/10
Healthy controls on a gluten containing diet	0/25	0/25	1/25	0/25	0/25	0/25

*p=0.0325 (Fisher's exact test) compared with IgG-AGA positives among CD/IgAD patients not on a strict gluten free diet (GFD). †p=0.0867 (Fisher's exact test) compared with IgG-AGA positives among CD/IgAD patients not on a strict GFD.

more than one year; (3) 10 coeliac patients with IgAD not on a strict GFD; (4) 11 untreated coeliac patients without IgAD; (5) 10 non-coeliac IgAD subjects on a gluten containing diet with normal intestinal mucosal findings; and (6) 25 control subjects, without IgAD, in whom a diagnosis of CD had been excluded (normal mucosa after a small intestinal biopsy). The last group included: (a) five healthy first degree relatives of coeliac patients; (b) 10 adult or paediatric patients with gastrointestinal diseases such as milk protein intolerance, postenteritis syndrome, Crohn disease, ulcerative colitis, or giardiasis; and (c) 10 healthy subjects who attended healthy child clinics.

Both original¹² and revised¹³ criteria for a diagnosis of CD were used. Assays of serum immunoglobulins, AGA, and IgA-EMA tests were performed as described previously.³ Serum IgA concentrations less than 0.05 g/l in the presence of normal levels of IgG and IgM was regarded as selective IgA deficiency. None of the patients who had a diagnosis of IgA deficiency was receiving pharmacological agents known to induce secondary IgA deficiency.¹⁴

IgG₁ ANTIENDOMYSIAL ANTIBODY DETECTION

Antiendomysial antibodies of the IgG₁ subclass (IgG₁-EMA), the most representative among the IgG subclasses, accounting for 70% in human serum,¹⁵ were determined in sera, diluted 1:5, by indirect immunofluorescence analysis, on cryostat sections of monkey oesophagus (Eurospital, Trieste, Italy). FITC conjugated antihuman IgG₁ (Sigma, St Louis, Missouri, USA) were used, diluted 1:100. Positive IgG₁-EMA results were identified by a fluorescent network around the smooth muscle fibres and evaluated blindly by two observers unaware of the clinical status of the patients. The agreement rate was 98.7%.

IgG ANTI-TISSUE TRANSGLUTAMINASE ANTIBODIES (IgG-ANTI-tTG) DETECTION

IgG-anti-tTG were detected using tTG coated 96 well plates (100 ng/well) from a commercially available IgA-anti-tTG detection kit (Immunopharmacology Research, Catania, Italy) activated with CaCl₂ (5 mM), as suggested by Sulkanen and colleagues⁴ and Dieterich and colleagues.⁷ After four washes with phosphate buffered saline (PBS) 0.15 M, 0.1% human serum albumin (HSA), and 0.05% Tween 20, the wells were blocked by incubation with HSA (2% in PBS) for two hours at room temperature. After three washes,

sera diluted 1:250 in PBS were incubated at room temperature for two hours. The presence of IgG-anti-tTG autoantibodies was evaluated after incubation with horseradish-peroxidase conjugated antihuman IgG (1:6000 in PBS, 0.05% HSA, one hour at room temperature) and substrate (1 mg/ml ortho-phenyldiamine (Sigma) in sodium citrate 1 M, citric acid 1 M, and 0.06% H₂O₂, 30 minutes at room temperature) as absorbance values of blocked reactions (0.3 M sulphuric acid) at 492 nm were measured on an ELISA reader. Sera were considered positive for IgG-anti-tTG when absorbance (ABS) of a sample was twofold greater than that of the calculated cut off value ((positive control ABS+negative control ABS)/2). Positive and negative control values were, respectively, absorbance of pool EMA-IgG positive and negative sera after background subtraction.

IgA-anti-tTG were measured with a kit (Immunopharmacology Research) following the manufacturer's instructions.

STATISTICAL ANALYSIS

Fisher's exact test was used to evaluate statistically significant differences among positive AGA, EMA, and anti-tTG antibody distributions using graphPAD InStat Algorithm (GraphPAD Software, San Diego, California, USA).

Results

Table 1 shows the results of AGA, EMA, and anti-tTG evaluations in different groups of CD and CD/IgAD patients, and in control subjects.

In untreated CD/IgAD patients, IgG-AGA, IgG₁-EMA, and IgG-anti-tTG were positive in all cases, whereas IgA-AGA, IgA-EMA, and IgA-anti-tTG were always negative. Coeliac patients with IgAD on a strict GFD did not produce AGA (IgA and IgG), EMA (IgA and IgG₁) or IgA-anti-tTG, whereas four were IgG-anti-tTG positive.

CD/IgAD patients not on a strict GFD were all IgG-AGA positive but, as detailed in table 2, 5/10 and 4/10 were IgG₁-EMA and IgG-anti-tTG negative, respectively.

Untreated coeliac patients without IgAD were AGA (IgA and IgG), EMA (IgA and IgG₁), and anti-tTG (IgA and IgG) positive.

IgAD subjects without coeliac disease and healthy controls were AGA (IgA and IgG), EMA (IgA and IgG₁), and anti-tTG (IgA and IgG) negative but three (two IgAD and one healthy control) were IgG-anti-tTG positive.

Table 2 Results of contemporary evaluation of antigliadin antibodies (IgG-AGA), antiendomysium antibodies (IgG-EMA), and antitissue transglutaminase antibodies (IgG-anti-tTG) in 10 patients with coeliac disease and selective IgA deficiency (CD/IgAD) not on a strict gluten free diet

No of patients	IgG-AGA	IgG-EMA	IgG-anti-tTG
4	Positive	Positive	Positive
3	Positive	Negative	Negative
2	Positive	Negative	Positive
1	Positive	Positive	Negative

Discussion

Recently, many attempts have been made to find a screening and diagnostic assay to identify those patients with IgAD who are also affected by CD. Until now, IgG-AGA have been the most widely used tool but we now know that it is not possible to totally exclude CD in IgAD patients if this test is negative³; therefore, there is a need for more reliable tests.

After identification of tissue transglutaminase (tTG) as the main antigen recognised by antiendomysium antibodies, ELISA tests have been developed to identify both IgG and IgA anti-tTG antibodies. The specificity of IgA-anti-tTG detection is very high and this test may be considered the best tool for identification and diagnosis of coeliac disease without IgAD. Unfortunately, results from IgG-anti-tTG tests have demonstrated that these antibodies are detectable not only in CD patients but also in sera obtained from subjects affected by autoimmune and inflammatory diseases.⁶ In contrast, IgG-EMA have been found in small amounts in CD patients with normal serum IgA^{5 16 17} showing high titres only in IgAD/CD patients.^{5 16 17 18} Similarly, high titres of Ig-anti-tTG antibodies were found in coeliac patients who were also affected by IgAD.^{4 7}

In a previous study³ we observed that in some conditions, IgG-AGA testing failed to identify CD in IgAD subjects. Based on the present data, both IgG-EMA and IgG-anti-tTG may be considered in the identification of CD in IgAD subjects as there were no false negatives in the group of newly diagnosed subjects. In particular, measurement of IgG₁-EMA seems to be highly sensitive (100%) and specific (100%) for a diagnosis of CD in subjects with IgAD allowing, in addition to IgG-AGA, identification of all coeliac patients among IgAD subjects. Based on IgG₁-EMA testing, no newly diagnosed CD/IgAD subject was negative for the IgG-anti-tTG test, even though two of 10 IgAD subjects without CD and one of 25 healthy controls were found to be IgG-anti-tTG positive. In this regard, even if the seminal papers on this topic have demonstrated that these antibodies are not specific for CD,^{4 6 7} follow up of these three subjects will clarify if they are latent or potential CD sufferers rather than false positives.

In addition, our findings showed that recognition of antiendomysium autoantigens induces IgG₁ antibody production at a level comparable with that observed for the IgA isotype. Indeed, we found IgG₁-EMA both in a group of untreated CD patients with normal serum IgA

levels and in a group of untreated CD patients with IgAD, whereas to date, IgG-EMA (evaluated as total IgG) have been found only in small amounts in CD patients with normal serum IgA^{5 16 17} and have been considered to have a compensatory role¹⁷ because of their unusually high titres in IgAD/CD patients.^{5 15 17 18} These discrepancies between our study and previous ones may be a result of technical differences. The immunofluorescence technique used to measure EMA antibodies is cumbersome and dependent on subjective interpretation. In addition, the variable sensitivity of IgG-EMA detection, which is influenced by type of tissue substrate used,⁵ may reduce the reliability of the IgG-EMA test. In contrast, identification of a subclass of IgG antibodies (that is, IgG₁, which represents more than 70% of all circulating IgG subclasses) may increase the sensitivity of the test in relation to improved reactivity of purified antihuman IgG₁ antibodies. In this respect, we cannot exclude the fact that preferential production of IgG₁ autoantibodies among IgG subclasses after gliadin sensitisation may occur in coeliac disease. A T helper (Th) 1-like immune response is predominant at the mucosal level in untreated coeliac patients with high production of interferon gamma,¹⁹ and it may be involved in the regulation of immunoglobulin production, conditioning the switch between different isotypes and subclasses.²⁰ In some pathologies (for example, Lyme borreliosis) a predominant interferon gamma immune response induces IgG₁ and IgG₃ antibody production instead of the IgG₂ or IgG₄ isotypes.²¹ If this is the case, a prevalent Th1-like cytokine response in coeliac disease may induce predominantly IgG₁ antibody production.

IgG-AGA and IgG-EMA, but not IgG-anti-tTG, disappeared in all IgA/CD patients on a strict GFD. Conversely, IgG-AGA were detectable in all IgAD/CD patients not on a strict GFD whereas there was an inconsistent pattern for IgG₁-EMA and IgG-anti-tTG antibodies. These results may depend on individual differences in compliance with a GFD. Furthermore, these findings seem to suggest that IgG₁-EMA and IgG-anti-tTG tests are not informative with regard to dietary compliance after gluten withdrawal. Perhaps they behave as IgA-EMA which, during dietary transgressions, become positive later than IgA and IgG-AGA,²² being positivity related to histological relapse rather than to gluten sensitivity. Clearly this topic deserves further study.

In conclusion, our data suggest that both IgG₁-EMA and IgG-anti-tTG are useful screening tests in CD, particularly for diagnostic purposes in IgAD subjects. Conversely, they are less satisfactory in evaluating dietary compliance with gluten withdrawal, and a multi-test protocol is recommended.

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Appendix

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