Do IgA anti gliadin and IgA anti endomysium antibodies show there is latent coeliac disease in primary IgA nephropathy?

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Abstract

The finding in primary IgA nephropathy of increased levels of IgA to food antigens and particularly to gliadin prompted the hypothesis that a subgroup of these patients may have latent coeliac disease. The observation that gliadin may experimentally induce IgA mesangial deposits supported this hypothesis. We evaluated specific immunological markers of coeliac disease (anti endomysium antibodies) which parallel histological changes of gluten sensitive enteropathy, and an IgA immunofluorescent test for anti gliadin antibodies in 18 patients with IgA nephropathy, in 56 untreated coeliac disease patients, in 254 controls (58 healthy and 196 disease controls). Anti endomysium antibodies were positive in 89.28% of coeliac patients, but negative in all IgA nephropathies and controls. IgA immunofluorescent test for anti gliadin antibodies, negative in all IgA nephropathy patients, was positive in 76.78% of coeliac patients and in 4.91% of controls. ELISA IgA anti gliadin antibodies were negative in controls, but positive in 22.22% of IgA nephropathy patients and in 60.71% of coeliac patients. Our data would suggest that in most patients with IgA nephropathy there is no evidence of latent coeliac disease.

Primary IgA nephropathy is a renal disease characterised by mesangial deposits containing IgA and, to a lesser extent, IgG and C3 which have electron dense features typical of immune complexes.¹ The antigens eliciting the IgA immune response that leads to IgA immune complex mesangial deposition are still unknown. Environmental, infectious, and food antigens could play a role in triggering the increase of IgA and IgA immune complex production.²

Among others, gliadin has, since the first report, been suggested as the association between coeliac disease and IgA nephropathy.³ Such an overt association is clinically infrequent and the role of gliadin was reconsidered only after the observation that a gluten free diet could modify some immunological abnormalities in some IgA nephropathy patients by reducing the concentrations of both circulating IgA immune complex⁴ and the titre of some dietary antigens.⁵ It was also shown that gliadin could experimentally induce IgA glomerulopathy in mice.⁶ Jejunal mucosa atrophy compatible with untreated coeliac disease has been rarely seen⁶⁻⁸ and intestinal mucosal permeability is not generally increased in IgA nephropathy⁹ as it is in coeliac disease.

The question arises, therefore, as to whether IgA nephropathy is associated with a subclinical form of coeliac disease or whether gliadin acts as an antigen, perhaps like other alimentary components, particularly those with lectinic properties.

The aim of this study was to evaluate in the sera of IgA nephropathy patients, the presence of new specific immunological markers of coeliac disease, shown to parallel histopathologic gut changes – that is, IgA class anti endomysium antibodies,^{10,13} and an IgA immunofluorescent test for antibodies to gliadin.^{14,15}

Methods

PATIENTS

The sera of 18 adult IgA nephropathy patients was studied whose disease was shown by prevalent IgA mesangial deposits in immunofluorescence on renal biopsy tissue; there were no laboratory or clinical signs of liver, intestinal, skin or systemic involvement.

Serum samples from 56 untreated adults with biopsy proven coeliac disease and from 58 healthy controls and 196 disease controls (Crohn's disease, ulcerative colitis, irritable bowel disease, food allergy, lymphoma, etc) were also studied. Sera from both patients and controls were stored at -20°C until tested.

IgA class anti endomysium antibodies (IgA-EmA) were detected by indirect immunofluorescence, on frozen sections of commercial slides of monkey oesophagus, provided by Biosystems, Italia. Sera were diluted 1:5 in phosphate buffered saline, pH 7.2, and were considered as positive if a thin fluorescent network around the smooth muscle fibres appeared. Immunofluorescent IgA class antibodies to gliadin were appraised on commercial slides of rat kidney (Biosystems, Italia) previously treated with gliadin, which selectively binds to reticulin fibres. Sera, diluted 1:10 in phosphate buffered saline pH 7.2, were considered as positive when originating periglomerular and peritubular fluorescence. Sera obtained from the patients and controls were read blind by three of us, and was 100% for anti endomysium antibodies and 97% for anti gliadin antibodies.

Serum anti gliadin antibodies of IgA and IgG isotypes were also assessed with an enzyme linked immunosorbent assay (ELISA) with microplates coated with wheat gliadin (Sigma) dissolved in carbonate/bicarbonate buffer at pH 9.6, as previously described.¹⁶ Values of absorbance index over mean + 2 (SD) of controls were considered as the cut off of positivity – that

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TABLE Results

	ELISA IgA-AGA	ELISA IgG-AGA	IF-IgA-Ema	IF-IgA-AGA
Controls:				
Healthy	0/58 (0%)	4/58 (6.89%)	0/58 (0%)	2/58 (3.44%)
Disease	0/196 (0%)	30/196 (15.30%)	0/196 (0%)	9/183 (4.91%)
Coeliac disease	34/56 (60.71%)	37/56 (66.07%)	50/56 (89.28%)	43/56 (76.78%)
(free diet)				
IgAGN	4/18 (22.22%)	0/18 (0%)	0/18 (0%)	0/18 (0%)

AGA=antigliadin antibodies; IF=immunofluorescence; EmaA=antiendomysium antibodies.

is, 0.200 absorbance index for IgA and 0.300 absorbance index for IgG class.

Results (Table).

IMMUNOFLUORESCENCE IgA ANTIENDOMYSIUM ANTIBODIES

Antiendomysium antibodies were found positive in 50 of 56 (89.28%) coeliac disease patients, but negative in all IgA nephropathy and in both healthy and disease controls.

IMMUNOFLUORESCENCE IgA ANTIGLIADIN ANTIBODIES

Positive staining for immunofluorescence IgA antigliadin antibodies was shown in 43 of 56 (76.78%) coeliac disease and in 11 of 241 (4.56%) controls, but in no IgA nephropathy patients. False positives were confined to patients with active small bowel Crohn’s disease.

ELISA IgA AND IgG ANTIGLIADIN ANTIBODIES

Higher than cut off limit titres of antigliadin antibodies IgA class were found in 34 of 56 (60.71%) coeliac disease patients, in four of 18 (22.22%) IgA nephropathy, but not in any controls. Antigliadin antibodies IgG titres resulted above the cut off limit in 37 of 56 (66.07%) coeliac disease patients, in 34 of 254 (13.38%) controls but in no IgA nephropathy patient.

False positives were found in four (6.89%) healthy controls, and in 30 (15.30%) patients (16 active Crohn’s disease, two food allergy, two irritable bowel disease, one lymphoma, five patients with other autoimmune disorders, and in four first degree relatives of coeliac disease patients).

Discussion

The detection of high titres of antigliadin antibodies in the sera of IgA nephropathy patients has drawn the attention of many authors to the possible relationship between IgA nephropathy and coeliac disease. Laurent’s group¹⁷ found a 70% frequency of high antigliadin antibodies titres in 27 IgA nephropathy patients by a single step ELISA and a 100% frequency by a more sensitive two step test.¹⁸ High IgA antigliadin antibodies titres were also reported by Nagy *et al*¹⁹ in 54% of 54 IgA nephropathy patients; these patients also showed high levels of other dietary antigens. In contrast, Fornasieri *et al*⁷ reported that only four of 121 (3%) of IgA nephropathy patients showed high antigliadin antibodies levels; Rostoker *et al*¹⁸ attributed this discrepancy to a difference in methods. They

coated ELISA plates with wheat gliadin in carbonate/bicarbonate buffer, while Fornasieri *et al*⁷ coated them in phosphate buffered saline, which allows glutenin but not gliadin extraction. Rodriguez-Soriano *et al*,²⁰ with the same method used by Laurent’s group,¹⁷ confirmed Fornasieri’s data:⁷ no antigliadin antibodies were detected in their patients. In a multicentre study,²¹ it was recently observed that IgA antigliadin antibodies had different prevalences among Italian, Australian, and Japanese IgA nephropathy patients by using ELISA with bicarbonate and alcohol gliadin extraction. Italian patients had a prevalence of IgA antigliadin antibodies in bicarbonate and ethanol ranging from 19 to 28.5%, Australian from 19 to 23.8% and Japanese from 0 to 16% only.

The contrasting data and the subsequent debate about several possibilities of interpreting antigliadin antibodies results, prompted us to extend our studies by using two new serological methods; the detection of antiendomysium antibodies and antigliadin antibodies by immunofluorescence, which are an improvement over the previous methods.^{10,15}

In our study, using the same method as Laurent *et al*,¹⁷ we detected high IgA antigliadin antibodies values in 22% of IgA nephropathy patients. No patient showed high titres of IgG antigliadin antibodies, that have proved to be more sensitive, albeit less specific,^{16,22,23} or positive for antiendomysium antibodies or immunofluorescence-IgA-antigliadin antibodies, whose specificity for gluten sensitive enteropathy is 100%.¹¹⁻¹⁴

False positive antigliadin antibodies have been reported previously, only for the IgG class, in gastrointestinal disorders (such as Crohn’s disease, ulcerative colitis, lymphoma, etc) and in autoimmune or bullous diseases,^{24,26} as in our own experience. On the contrary, IgA class antigliadin antibodies have been considered specific for gluten sensitive enteropathy.^{16,27,28} Yet, the discrepancy with immunofluorescence IgA antigliadin antibodies and antiendomysium antibodies suggests that this positivity is not specific for gluten sensitive enteropathy in IgA nephropathy.

Immunofluorescence IgA antigliadin antibodies, which detect specific gluten sensitivity rather than coeliac disease, were negative in all IgA nephropathy patients. As previously reported,¹⁴ we also found that false positives accounted for active Crohn’s disease with small intestinal involvement, possibly reflecting an increased permeability to food antigens.

As six of our IgA nephropathy patients (three of whom were ELISA IgA antigliadin antibodies positives) previously underwent jejunal biopsy with no histological evidence of coeliac disease,⁴ and as none showed either antiendomysium antibodies or immunofluorescence antigliadin antibodies positivity, we did not consider it ethical to extend this invasive procedure to the remaining patients. Screening tests for gluten sensitive enteropathy cannot replace biopsy as a definitive diagnostic tool; nonetheless those tests which only occasionally yield a false negative are useful in deciding which person to biopsy when the diagnosis of coeliac disease is involved.

On the other hand, both Fornasieri *et al*⁷ who found villous atrophy in two of 141 and Stevens *et al*⁸ who, of 31 IgA nephropathy patients all submitted to intestinal biopsy, found subatrophy in only one, confirmed the absence of a strong association between gluten sensitive enteropathy and IgA nephropathy.

In conclusion, the absence of IgA anti-endomysium antibodies and immunofluorescence IgA antigliadin antibodies in 18 IgA nephropathy patients would show that in most patients with IgA nephropathy no association with latent coeliac disease exists. Gliadin plays a role in IgA nephropathy, perhaps mediated by its lectinic properties that favour the binding to cultured mesangial cells.²⁹ This has been shown both by the formation of IgA deposits in mice orally immunised with gliadin and by the decrease of levels of IgA to food antigens and of IgA immune complex in a group of patients with IgA nephropathy. IgA nephropathy does not, however, seem to be a dermatitis herpetiformis of the kidney as has been previously suggested.⁸

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