Do adults with high gliadin antibody concentrations have subclinical gluten intolerance?

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Abstract

Gliadin antibodies of the IgG and IgA isotypes and IgG subclasses were measured in 200 adults who were randomly selected from the Icelandic National Register. Those with the highest gliadin antibody concentrations were invited with negative controls to participate in a clinical evaluation. Neither the study subjects nor the physicians who recorded and evaluated the clinical findings were aware of the antibody levels. Significantly higher proportion of the gliadin antibody positive individuals reported unexplained attacks of diarrhoea ($p=0.03$), and IgA gliadin antibodies were associated with increased prevalence of chronic fatigue ($p=0.0037$). The gliadin antibody positive group also showed significantly decreased transferrin saturation, mean corpuscular volume and mean corpuscular haemoglobin compared with the gliadin antibody negative controls. Serum folic acid concentrations were

significantly lower in the IgA gliadin antibody positive individuals. On blind global assessment 15 of the 48 participants were thought to have clinical and laboratory features that are compatible with gluten sensitive enteropathy, and 14 of these were in the gliadin antibody positive group ($p=0.013$). Complaints that have not been associated with gluten intolerance had similar prevalence in both groups with the exception of persistent or recurrent headaches that were more common in the gliadin antibody positive group. These findings raise the possibility that a subclinical form of gluten intolerance may be relatively common.

Gluten sensitive enteropathy includes a wide spectrum of symptoms, and the clinical manifestations can be subtle.¹ Recent studies have indicated that this disease may be more common than previously recognised, and that it may often present with chronic fatigue and non-specific abdominal complaints.^{2,3} It has further been suggested that inappropriate immunity to gliadin without histological changes in the gut mucosa may be a fairly common latent form of gluten sensitive enteropathy that only occasionally progresses to the second stage of typical coeliac disease.⁴ Gliadin antibodies can be detected in the serum of 82-96% of patients with coeliac disease and are also frequently found in patients with dermatitis herpetiformis.⁵⁻⁷ The pathogenic significance and diagnostic value of these antibodies is uncertain,^{4,8} but it has been suggested that measurement of serum gliadin antibodies may help to select patients for intestinal biopsy^{5,7,9,10} which remains the only reliable diagnostic procedure for gluten sensitive enteropathy. Changes in the jejunal mucosa, however, may range from increase in the numbers of interepithelial lymphocytes, through abnormal lymphocytic infiltration of the lamina propria and crypt hyperplasia, to a flat mucosa.⁸ Patients with gluten sensitive diarrhoea but normal villous appearance have also been reported.¹¹ We and others have found that there is a considerable overlap in serum gliadin antibodies concentrations between patients with untreated but proven gluten intolerance and control subjects (Jónsdóttir *et al* unpublished data).⁸ It was therefore of interest to evaluate clinically and compare randomly selected individuals with undetectable and high serum gliadin antibodies concentrations.

Methods

SELECTION OF STUDY SUBJECTS

The Figure shows the overall design of the study.

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Study design

In 1985 serum samples were obtained from 400 adults aged 30–50 years, who were selected at random from the National Register in Iceland. The samples were aliquoted and stored for determination of reference distribution for various assays in our laboratory. From this collection 200 samples were randomly selected and used to establish the distribution of gliadin antibodies in adults. This distribution was skewed and the upper limit of normal was therefore set at the 95% level for each GA isotype and IgG subclass (Jónsdóttir *et al* unpublished data). Of these 200 individuals all those with IgA-GA, total IgG-GA or one or more IgG-GA subclass above the 95% level were selected for clinical assessment. Because some had more than one gliadin antibodies isotype or IgG subclass above this limit, only 32 individuals qualified for the antibody positive group of this study. As negative controls, 20 age and sex matched individuals were selected from those who did not have any detectable gliadin antibodies isotype or subclasses. The age, sex, and attendance rate of the gliadin antibody positive and negative groups is shown in the Figure.

CLINICAL ASSESSMENT

The study was approved by the Ethical Committee of the National University Hospital and each participant received a letter explaining the objectives of the study and the procedures involved. Four declined to participate, and the study group therefore consisted of 31 gliadin antibodies positive and 17 gliadin antibody negative individuals (Figure). A structured questionnaire was designed for the clinical assessment, and all the participants answered this with the help of the same physician. This questionnaire was primarily designed for assessing symptoms and signs of malabsorption such as abnormal fatigue, anaemia, weight loss, diarrhoea, and other gastrointestinal symptoms, but an attempt was also made to evaluate the general health of the participants with the help of a visual analogue scale. Medical treatments and hospitalisations were also recorded as were skin disorders and over 20 different symptoms that have not been associated with gluten intolerance including headaches, musculoskeletal pains, joint pains and a variety of psychosomatic complaints. Neither the study subjects nor the physician knew about the gliadin antibodies findings.

MEASUREMENT OF GLIADIN ANTIBODIES

An ELISA (enzyme linked immunoadsorbent assay) system was used as described in detail elsewhere (Jónsdóttir *et al* unpublished data). Briefly, Dynatech microtitre plates were coated with crude gliadin (Sigma) dissolved in 60% alcohol. Duplicates of the serum samples diluted 1:50 in phosphate buffered saline – Tween were incubated at room temperature for five hours followed by alkaline phosphatase conjugated antihuman IgG or IgA over night. Gliadin antibodies of the individual IgG subclasses were determined by incubation with subclass specific monoclonal antibodies followed by alkaline phosphatase conjugated rabbit antimouse

immunoglobulin that had been absorbed with human immunoglobulin. For each isotype and subclass the absorbance of the serum samples was read against a dilution curve of an internal standard containing 100 arbitrary units of gliadin antibodies for each isotype and IgG subclass. This standard was prepared by pooling sera from patients with high concentrations of gliadin antibodies of all isotypes and IgG subclasses. Intra and interassay variability was approximately 8% and 15% respectively. The distribution of gliadin antibody concentrations was skewed in 200 randomly selected adults, and the upper limit of normal was therefore set at the 95% level for each isotype and IgG subclass.

STATISTICAL ANALYSIS

Statistical analysis was done with the aid of Statistical Package for the Social Sciences (SPSS/PC+) program. The recorded parameters were compared for the gliadin antibodies positive and negative groups using both parametric (Student's *t* test) and non-parametric (Mann-Whitney U-test) analysis. χ^2 with Yates's correction was used for binominal variables.

Results

The two serum samples that were obtained from each participant at an interval of three years showed remarkably similar gliadin antibody concentrations and isotype and IgG subclass patterns. Thus, 30 of the 31 gliadin antibody positive individuals were still above the 90% level and 23 remained above the 95% level for the particular gliadin antibody types that their selection into the study was based upon. Similarly, none of the 17 gliadin antibody negative participants had detectable gliadin antibody activity after three years. The clinical analysis could therefore be based upon the original gliadin antibody findings.

None of the study subjects had typical features of severe malabsorption. A significantly higher proportion of the gliadin antibody positive participants claimed to have had unexplained attacks of diarrhoea ($p=0.03$), however. There was also increased prevalence of chronic fatigue in the IgA-gliadin antibody positive individuals ($p=0.0037$), but participants with IgG-gliadin antibodies did not report this complaint more often than the gliadin antibody negative controls. The overall prevalence of skin problems was not increased in the gliadin antibody positive participants. Two of the gliadin antibodies positive subjects, however, had history of pruritic vesicular skin eruptions on extensor sites. They had both been treated by dermatologists and did not have any skin eruptions at the time of the study. Skin biopsies were not carried out. Nine of the study subjects, seven of whom were gliadin antibody positive, complained of chronic or recurrent headaches. Four had classical migraine and they were all gliadin antibody positive. None of the other recorded complaints were more common in the gliadin antibody positive group.

As shown in Table I several significant differences were observed in haematological

parameters between the two groups, collectively indicating that the gliadin antibody positive individuals had lower iron stores than the gliadin antibody negative controls. Furthermore, folic acid concentrations were lower in the gliadin antibody positive participants and this was significant for the IgA-gliadin antibody positive subjects ($p=0.007$). Interestingly, those individuals who were positive for both IgA and IgG gliadin antibodies showed the lowest values for mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and iron and highest total iron binding capacity, while participants with isolated IgA-gliadin antibodies or IgG-gliadin antibodies rises, although lower, did not differ significantly from the negative controls in these haematological parameters (Table II). Although some of these haematological parameters have different normal distribution in men and women, separate analysis of each sex gave differences which were similar to those presented in Table I, even when the results were analysed by a non-parametric test. This is presumably because the sex ratio was similar in both groups. The non-parametric ranking test, however, gave higher significance values than those shown in Table I.

Three women who were all gliadin antibody positive had a mild iron deficiency anaemia associated with low ferritin and raised total iron binding capacity. Hypochrome anaemia was not observed in any of the gliadin antibody negative controls.

The results of a blind global assessment, by a gastroenterologist, of each study subject for clinical and laboratory features of gluten sensitive enteropathy are shown in Table III. None of the participants were considered to be likely to have this disease. Of the 15 who had features that were thought to be consistent with this diagnosis, however, all but one were gliadin antibody positive ($p=0.013$).

The clinical significance of a particular antibody pattern or rise in a single gliadin antibody isotype or subclass could generally not be analysed statistically because there were too few individuals in each group.

Discussion

This study was organised in order to evaluate the significance of high serum concentrations of gliadin antibodies in individuals who do not have definite clinical features of gluten intolerance.

Measurements at an interval of three years showed that the levels and patterns of these antibodies were remarkably stable in the study subjects. As our original study group involved only 200 randomly selected adults it is not surprising that no case of overt symptomatic coeliac disease was found. The gliadin antibody positive test subjects, however, were more prone than the controls to have episodes of diarrhoea, fatigue and haematological features that are indicative of decreased iron storage. Furthermore, when all relevant findings for each study participant were assessed globally 14 of the 15 individuals with features considered compatible with gluten sensitive enteropathy were in the antibody positive group.

Recent studies have provided strong indirect support for the notion that immune reactions against gluten components may play a key role in the pathogenesis of coeliac disease.¹² There is increasing evidence, however, that the mucosal damage is mostly mediated by T cells,^{13,14} and antibodies to gluten components may largely be epiphenomenal, either indirectly reflecting aberrant T cell responses to gluten or increased permeability of the gut mucosa. Indeed, a proportion of adult patients with proven gluten intolerance have been found to be persistently negative for both IgG and IgA antibodies to crude gliadin (Jónsdóttir *et al* unpublished data), but some patients with low serum antibody levels may have high levels of secretory IgA or IgM anti gliadin in jejunal aspirates.⁴

We know of only one attempt to study the incidence of gluten sensitive enteropathy using measurement of gliadin antibodies in serum as a screening procedure.¹⁵ Sera from 1866 blood donors were tested and all those above the 3% limit for IgA gliadin antibodies were offered jejunal biopsy. Of 43 who underwent this examination seven had mucosal changes consistent with gluten sensitive enteropathy, suggesting a prevalence of 3.7 per 1000 in apparently

TABLE I Comparison of haematological parameters in GA positive and GA negative individuals

	Mean values (SD)		p
	Gliadin antibody positive (n=31)	Gliadin antibody negative (n=17)	
RBC (10 ¹² /l)	4.7 (0.35)	4.4 (0.52)	NS
Hb (g/l)	140.3 (15.55)	139 (8.98)	NS
Hct (l/l)	0.426 (0.049)	0.414 (0.414)	NS
MCV (fl)	90.6 (6.92)	95.5 (3.52)	0.008
MCH (pg)	29.9 (2.65)	32.1 (1.29)	0.048
MCHC (g/l)	329.3 (7.98)	335.6 (7.64)	0.002
S-Iron (μmol/l)	14.7 (6.8)	18.3 (6.1)	NS
TIBC (μmol/l)	60.7 (10.2)	53.7 (9.2)	0.024
Transferrin sat (%)	25.3 (12.2)	34.9 (13.1)	0.01
Ferritin (μg/l)	139.6 (117.8)	117.5 (106.5)	NS
B12 (pmol/l)	328.4 (113.3)	368.3 (111.1)	NS
S-Folate (nmol/l)*	9.8 (6.34)	12.8 (9.43)	NS

*Serum folate concentrations were significantly lower in IgA gliadin antibody positive individuals, 7.5 nmol/l and 11.6 nmol/l, respectively ($p=0.007$).

TABLE II Comparison of haematological parameters in individuals with elevations in single or two gliadin antibody isotypes

	Rise in:		
	IgA only	IgG only	IgA and IgG
RBC (10 ¹² /l)	4.67	4.63	5.18
MCV (fl)	91.5	91.5	84.6
MCH (pg)	30.2	30.3	27.3
MCHC (g/l)	329	331	321
Iron (μmol/l)	15.0	14.8	14.0
TIBC (μmol/l)	64.8	57.3	68.2

RBC=red blood cell count; MCV=mean corpuscular volume; MCH=mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration; TIBC=total iron binding capacity.

TABLE III Blind global assessment of the study subjects

Findings	Gliadin antibody positive	Gliadin antibody negative
Highly suggestive of GSE*	0	0
Compatible with GSE	14	1
Not suggestive of GSE	17	16

*Gluten sensitive enteropathy. $p=0.013$ (Yates's correction).

healthy Swedish blood donors. None of these seven had clinical features that would have justified jejunal biopsy, but the four who went on a gluten free diet all showed a remission of the mucosal lesions.¹⁵

Our study only involved 200 randomly selected adults and 14 of 31 with relatively high concentrations of IgG and/or IgA gliadin antibodies had clinical features that were thought to be compatible with gluten sensitive enteropathy. It is of course unlikely that definite histological features of coeliac disease will be found in all these individuals, although they have clearly mounted an immune response to gliadin, and may therefore have latent gluten intolerance.⁴ It is conceivable that abnormal immunological activity in the gut mucosa^{4,14} may affect the uptake of dietary folate or iron before any villous flattening has occurred. A further comparative study of apparently healthy gliadin antibody positive and negative individuals is therefore justified, involving measurement of gut permeability, gliadin antibodies in jejunal aspirates, immunohistological evaluation of gut biopsies and histocompatibility locus antigen typing.

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