

## Humoral response to $\alpha$ gliadin as serological screening test for coeliac disease

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**SUMMARY** The diagnostic value of measuring  $\alpha$  gliadin antibodies in children with suspected coeliac disease has been evaluated prospectively. Jejunal biopsy and  $\alpha$  gliadin antibody measurements were performed in 77 consecutive children who were being investigated for a suspected malabsorption syndrome. The typical small intestinal histological lesion of coeliac disease was found, and this diagnosis was subsequently confirmed clinically in 20 children. Raised IgG  $\alpha$  gliadin antibody concentrations were found in 19 (95%). Fifty of 57 patients (88%) with a normal jejunal mucosa had normal  $\alpha$  gliadin antibody concentrations. These results are similar to those previously reported in a prospective study of adult patients with coeliac disease and indicate that measurement of  $\alpha$  gliadin antibody is a highly sensitive and specific screening test for childhood coeliac disease.

The gluten sensitive enteropathy characteristic of coeliac disease is thought to be caused by an abnormal immunological reaction to wheat protein.<sup>1</sup> Previous studies in adult patients with coeliac disease have shown both cellular<sup>2,3</sup> and humoral<sup>4</sup> sensitisation to  $\alpha$  gliadin, a purified fraction of wheat protein. In a prospective study of 90 adult patients  $\alpha$  gliadin antibody concentrations were raised in 82% of 44 patients with untreated coeliac disease, whereas normal antibody concentrations were found in 87% of 46 patients with normal jejunal mucosa.<sup>4</sup> This study has now been extended to include a total of 206 patients and measurement of  $\alpha$  gliadin antibody has continued to show a specificity of 88% and sensitivity of 81%. The present study assessed the value of  $\alpha$  gliadin antibody measurement in the diagnosis in children.

### Patients and methods

**Prospective study: patients and controls.** Children who presented with symptoms suggestive of a malabsorptive disease were assessed. A consecutive group of 77 children undergoing a jejunal biopsy examination were studied without knowledge of their clinical or histological state. Their ages ranged from 9 months to 15 years, with a median value of 6 years. IgG  $\alpha$  gliadin antibody concentrations were measured and correlated with their jejunal his-

tology. Twenty children had coeliac disease diagnosed on the basis of their jejunal histology and clinical symptoms while on a normal diet, and all 20 responded clinically to a gluten free diet. At diagnosis, 16 biopsy specimens had subtotal villous atrophy, and the remaining four had partial villous atrophy. Ten of these children were subsequently re-exposed to gluten; clinical deterioration accompanied this challenge and the biopsy specimens again showed changes consistent with coeliac disease. The remaining 57 children had a normal small intestinal mucosa.

IgA  $\alpha$  gliadin antibody concentrations were measured in 12 of the 20 patients, and both IgG and IgA antibody concentrations to soya protein extract and  $\beta$  lactoglobulin were also measured in 12 children with confirmed coeliac disease and in 31 children with normal jejunal biopsy specimens.

**Establishment of IgG and IgA  $\alpha$  gliadin antibody concentrations in normal children.** Serum samples were obtained from 54 control children, whose ages ranged from 1 to 15 years, with a median age of 7 years. These children had been admitted to hospital for non-gastrointestinal disorders and were assessed for specific IgG  $\alpha$  gliadin antibody concentrations, which were correlated with their age. IgA  $\alpha$  gliadin antibody concentrations were also assessed in 45 of these subjects.

**Serum  $\alpha$  gliadin antibody detection.** Serum antibody concentrations were measured by enzyme linked immunosorbent assay (ELISA) as previously described.<sup>4</sup> The results are expressed as ELISA indices (EI), where:

$$EI = \frac{\text{Mean of three originally derived readings of the test sample}}{\text{Mean of three originally derived readings of the background}}$$

**Antigens.**  $\alpha$  gliadin was prepared from gliadin by ion exchange chromatography on a carboxymethylcellulose column as described by Gehrke *et al.*<sup>5</sup> Soya protein extract was prepared by 70% ethanol extraction from soya flour, as described for the preparation of alcohol soluble gliadin proteins from crude gliadin.<sup>6</sup>  $\beta$  Lactoglobulin was obtained from Sigma.

**Statistics.** The data were analysed by the Wilcoxon rank sum test.

**Results**

**$\alpha$  Gliadin antibody concentrations in normal control**

**children.** The IgG and IgA  $\alpha$  gliadin antibody concentrations found in the control children are shown in Figure 1. The concentrations gradually increased with age and reached adult values at about 12 and 7 years of age, respectively. The increase was more pronounced in the IgG class  $\alpha$  gliadin antibody.

**Prospective analysis of  $\alpha$  gliadin antibodies in children presenting with a possible malabsorptive disease.**

In 77 children who underwent jejunal biopsy examination coeliac disease was diagnosed in 20, 19 of whom had raised concentrations of IgG  $\alpha$  gliadin antibody (sensitivity 95%) (Fig. 2, Table). The remaining 57 children had a histologically normal small intestinal mucosa: of these, 50 had normal  $\alpha$  gliadin antibody concentrations (specificity 88%). IgA  $\alpha$  gliadin antibody concentrations were also estimated in 12 of the 20 children with coeliac disease (Fig. 3). In the untreated group with coeliac disease, although the mean (SD) concentration (2.09 (1.35)) was significantly raised over the mean (SD) of the control group (1.28 (0.50)) ( $p < 0.05$ ), only five of the 12 patients tested fell above the normal range.

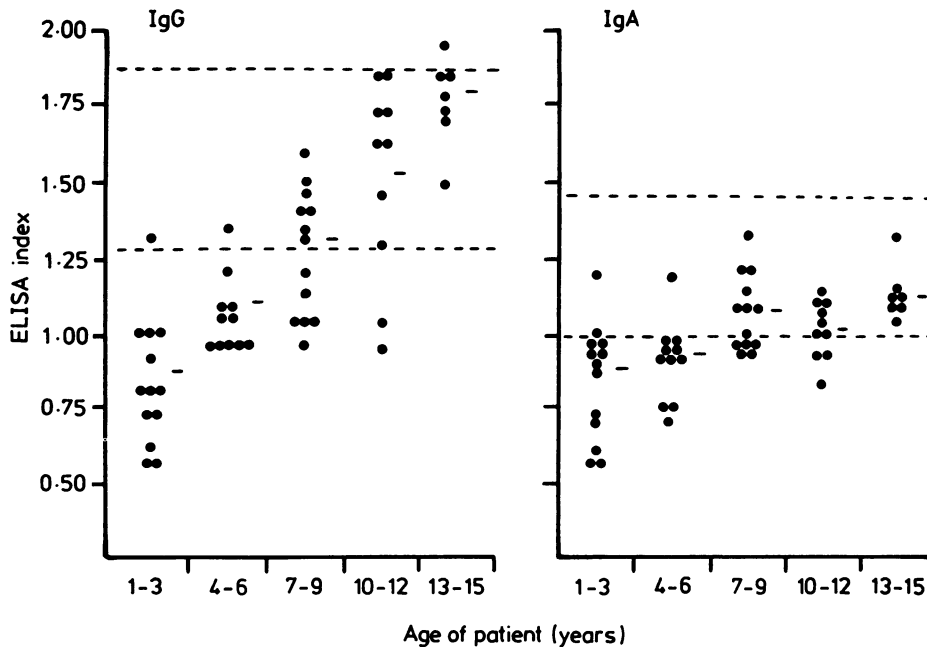


Fig. 1 Variation in IgG and IgA  $\alpha$  gliadin antibody concentrations with age in the normal population. Results expressed as mean (SE) enzyme linked immunosorbent assay (ELISA) index. The solid black lines indicate the mean (SD) normal adult value.

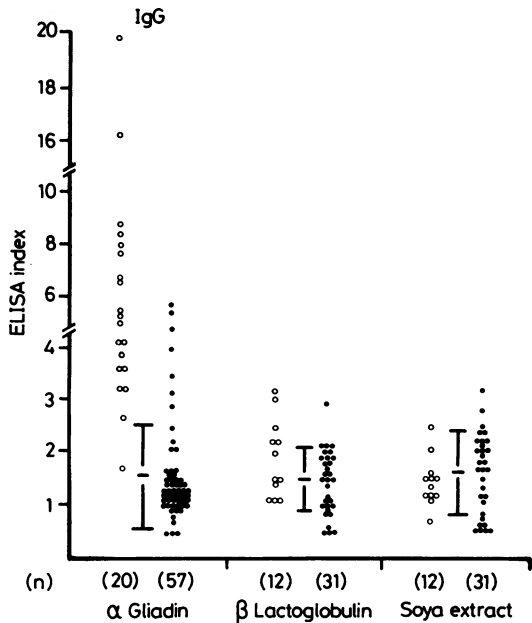


Fig. 2 IgG antibody concentrations to  $\alpha$  gliadin,  $\beta$  lactoglobulin, and soya protein extract in untreated patients with coeliac disease (○) and control subjects (●). The bars indicate the mean (SD) enzyme linked immunosorbent assay (ELISA) index of the control group.

Table Correlation between IgG  $\alpha$  gliadin antibody results and mucosal histology in 77 children

	Biopsy result	
	Positive	Negative
$\alpha$ Gliadin antibody result:		
Positive	19	7
Negative	1	50
Total	20	57

Sensitivity (probability of detecting coeliac disease when the test result is positive)=95%; specificity (probability of excluding the disease when the result is negative)=87.7%.

**Incidence of antibodies to other dietary antigens in children with coeliac disease and normal children.**

The results for IgG and IgA concentrations to soya protein extract and  $\beta$  lactoglobulin in 12 children with coeliac disease proved by biopsy examination and 31 controls are shown in Figures 2 and 3, respectively; the  $\alpha$  gliadin results are included for comparison. Using  $\beta$  lactoglobulin, the mean (SD) IgG antibody concentration (1.98 (0.70)) of the untreated group with coeliac disease was raised above the mean (SD) of the control subjects (1.507 (0.593)). The difference between the two groups

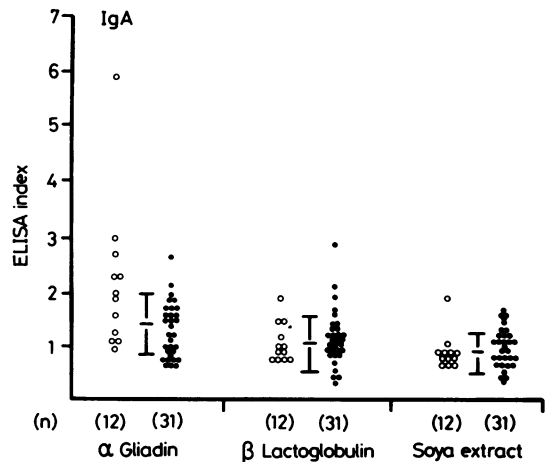


Fig. 3 IgA antibody concentrations to  $\alpha$  gliadin,  $\beta$  lactoglobulin, and soya protein extract in untreated patients with coeliac disease (○) and control subjects (●). The bars indicate the mean (SD) enzyme linked immunosorbent assay (ELISA) index of the control group.

was not significant, however, and although four of the patients with coeliac disease had raised titres, four of the controls who were being investigated for gastrointestinal symptoms also had raised antibody concentrations. No difference was seen between the IgG concentrations to soya protein extract in either the group with coeliac disease or the control group.

Detection of specific IgA antibodies to  $\beta$  lactoglobulin and soya protein antigens showed no difference in the response of the untreated children with coeliac disease and the controls.

**Discussion**

This prospective study shows the diagnostic value of measuring  $\alpha$  gliadin antibody in children with symptoms of malabsorption and in whom coeliac disease is suspected. When 77 consecutive children were investigated 20 had the typical small intestinal histological lesion of coeliac disease, 19 of whom had raised IgG  $\alpha$  gliadin antibody concentrations, whereas 50 of the 57 children with normal small intestinal histology had normal antibody concentrations. All of the 20 children with the coeliac lesion showed improvement clinically on gluten free diet, and the 10 children who were subsequently challenged with gluten showed a clinical relapse, which was histologically confirmed on further biopsy examination. This gave the test a sensitivity of 95% and specificity of 88%, and these values agree well with our previous prospective study of adults with

suspected coeliac disease.<sup>4</sup> The results also confirm the findings of Burgin-Wolff *et al*, who described the only other study in which measurement of wheat protein antibodies was prospectively evaluated as a screening test in childhood coeliac disease.<sup>7</sup> They employed gliadin as antigen in a fluorescence assay system and found raised antibody concentrations in all their patients with coeliac disease and in 16% of those without coeliac disease. The two studies further concur in that measurement of IgG class antibodies was most informative and that antibody concentrations to other dietary antigens were of no diagnostic help.

In previous non-prospective studies of coeliac disease an increased incidence of IgG antibodies to wheat protein antigens was shown.<sup>8-11</sup> As wheat protein antibodies were also found in other diseases, however, these assays could not be relied on to discriminate consistently between patients with coeliac disease and those without. Consequently, many investigators have recommended the measurement of IgA wheat protein antibodies.<sup>10-13</sup> Although the finding of wheat protein antibodies of IgA class is generally quite specific for the diagnosis of coeliac disease, several reports show this to be an insensitive test, especially in older children and adults.<sup>10 11 13</sup> In this study, although IgA  $\alpha$  gliadin antibodies were found in fewer of the patients without coeliac disease, raised concentrations were detected in only five of the 12 serum samples of patients with coeliac disease, rendering the detection of IgA  $\alpha$  gliadin antibodies unsuitable as a screening test.

The antigen used in our assay was  $\alpha$  gliadin. There is evidence that the major cellular and humoral immune response in patients with coeliac disease is directed towards this particular subfraction of gliadin<sup>3 14 15</sup> and that it is more toxic to coeliac jejunal tissue than other wheat protein fractions.<sup>16-18</sup> Unfractionated gliadin used in this ELISA system did not discriminate adequately between patients with and without coeliac disease.<sup>4</sup> As unfractionated gliadin was used in previous studies this may explain the difficulties experienced in differentiating the patient group from the control group,<sup>8 13</sup> although in some assays gliadin can be a satisfactory test antigen.<sup>7</sup>

To determine the effect of age on  $\alpha$  gliadin antibody responses, samples from normal control children aged 1 to 15 years were evaluated. A gradual increase in both IgG and IgA  $\alpha$  gliadin antibody concentrations was found that paralleled the age related increase in total immunoglobulin concentrations. The use of age matched controls was not necessary when screening for coeliac disease, however, as the titres obtained from the children

with coeliac disease exceeded even the normal adult values. Raised antibody concentrations were found in seven of the control subjects. Of these, one patient had cystic fibrosis, two had a strong family history of coeliac disease, one had cow's milk protein intolerance, one was less than the 25th weight and 50th height centiles and had diarrhoea, and the remaining two had persistent diarrhoea. It has been documented that patients with coeliac disease vary in their sensitivity to gluten and may require a high gluten intake for several months before showing histological changes.<sup>19</sup> Gluten challenge might therefore induce the characteristic lesion of coeliac disease in some of this group with raised  $\alpha$  gliadin antibody concentrations.

Screening tests for coeliac disease, including measurement of faecal fat, measurement of D-xylose absorption, radiological examination of the small bowel, haematological investigations, and detection of reticulin antibodies have been used in the past. None of these investigations is regarded as adequately sensitive or specific for coeliac disease.<sup>20-22</sup> In contrast, our study shows that as only one patient with true coeliac disease had normal antibody concentrations measurement of  $\alpha$  gliadin antibody using ELISA in children with suspected coeliac disease is a highly sensitive test. As this test is simple and cheap to perform it is useful for screening patients before performing the ultimate investigation—that is, small intestinal biopsy. The assay may also help to distinguish patients likely to respond to gluten withdrawal from those with other malabsorptive diseases. Finally, as gluten withdrawal is accompanied by a fall in antibody concentrations<sup>4 7</sup>  $\alpha$  gliadin antibody measurement may be used to monitor dietary compliance.

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