Salivary IgA antigliadin antibody as a marker for coeliac disease

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Abstract

In recent years, serum antibodies to gliadin (AGA) have been reported to be useful markers of coeliac disease. IgA AGA have also been found in intestinal secretions and saliva in coeliac disease and may offer a convenient, non-invasive screening test. In order to test this hypothesis, salivary and serum AGA were measured in children with coeliac disease proved by biopsy and compared with several control groups. Measurement of salivary IgA AGA provided excellent discrimination between those children with coeliac disease and the control groups, and our study suggests that it may provide a rapid, non-invasive method of screening for this disease before intestinal biopsy.

Coeliac disease remains an important cause of malabsorption in children. Definitive diagnosis requires histological confirmation by jejunal biopsy showing subtotal villous atrophy, increased numbers of intraepithelial lymphocytes, and crypt cell hyperplasia. Small intestinal biopsy is invasive, involves a period of fasting, and requires exposure to radiation to confirm correct positioning of the capsule. Many children undergo biopsies that subsequently prove to be negative, and thus various non-invasive tests have been proposed as screening tests. Tests such as xylose absorption and differential sugar permeability lack specificity and may be falsely positive in children with other causes of small intestinal mucosal damage.¹ Recently, attention has been directed towards the measurement of serum antibodies to gliadin,²⁻⁹ smooth muscle endomysium,⁸⁻¹² reticulín,¹²⁻¹⁴ and human jejunum,¹⁵ with different workers finding varying degrees of specificity and sensitivity for these tests. Antigliadin antibodies (AGA) are also known to be present in the intestinal secretions and saliva of patients with coeliac disease¹⁶⁻²⁰ and measurement of these might therefore provide a useful screening test.

A preliminary study reported in Cardiff suggested that measurement of salivary IgA AGA might be a reliable marker for coeliac disease in adults.²¹ Such a screening test would have obvious potential benefits in childhood and might reduce the need for more invasive tests involving blood sampling. The aim of this present study was to determine the usefulness of the test in childhood and to compare the results with serum measurements of IgA and IgG AGA.

Patients and methods

A prospective study was designed to determine

the prevalence of salivary and serum IgA AGA and serum IgG AGA in groups of children with coeliac disease, other gastrointestinal disorders, cystic fibrosis, and in children with no evidence of gut disease.

PATIENTS

There were 141 patients, divided into five groups, in whom AGA were measured. In group A were 19 patients (age range 9 months-14 years, median 5 years) who had coeliac disease proved by biopsy: they comprised 16 studied at presentation and three after gluten challenge. In group B were nine children (age range 2-18 years, median 6 years) who had established coeliac disease and were clinically well on a gluten free diet. Both these groups had satisfied the revised European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) criteria for diagnosis.²² In group C were 60 children (age range 6 months-15 years, median 4 years) undergoing jejunal biopsy who subsequently proved to have conditions other than coeliac disease (such as cows' milk or soy protein intolerance or toddler diarrhoea). In group D were nine children (age range 1-13 years, median 9 years) with cystic fibrosis. In group E were 44 children (age range 6 months to 18 years, median 4 years) with no clinical evidence of gastrointestinal disease who were having blood tests for other reasons. Small intestinal histology was not available for the last two groups of children.

It proved impossible to collect both serum and saliva in every patient in each group and the results represent all the data available in each group of patients. Simultaneous saliva and serum data were, however, available in eight patients. The district ethical committee had approved the study and consent was obtained from all parents and from older children if possible.

METHODS

Whole unstimulated saliva was collected from the patients. Older children were able to spit directly into sterile containers and, for younger children and infants, a suction catheter attached to gentle wall suction was used to obtain the specimens. At least 1 ml was obtained from each child. The saliva was heated at 56°C for 30 minutes to inactivate complement and enzyme activity, and centrifuged at 1000 *g* for 20 minutes to remove particulate matter. The supernatant was stored at -20°C until the assays were performed. Salivary IgA AGA were

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assayed using a standard enzyme linked immunosorbent assay (ELISA) technique as previously described.²¹

For the serum assays, a commercial gliadin IgA ELISA test kit (Pharmacia) was used and a similar ELISA technique (in another laboratory) was used to measure serum IgG AGA. The results for each assay were expressed in optical density units and the results of saliva and serum antibodies from each group were compared and analysed by non-parametric means using the Mann-Whitney U test.

Results

Table 1 shows the numbers of patients studied and the median IgA AGA values for each group with fig 1–3 showing the individual results.

Salivary IgA AGA (fig 1)

It is clear that AGA concentrations were significantly higher in patients with untreated coeliac disease compared with the patients in the other groups ($p<0.0001$ in each case). All patients with untreated coeliac disease had salivary IgA AGA concentrations >0.31 optical density units. Using this value as an arbitrary limit to achieve 100% sensitivity for the test, there were seven values >0.31 in the other groups (false positive values), giving the test a specificity of 90%. In these seven children, the jejunal histology showed partial villous atrophy in four (clinical diagnosis of cows' milk protein intolerance in each case) and was normal in three (clinical diagnosis of toddler diarrhoea in each case).

Table 1 Salivary IgA AGA

	No studied	Optical density units	
		Range	Median
(A) Untreated coeliac disease	17	0.309–1.894	0.594
(B) Coeliac disease on gluten free diet	6	0.039–0.252	0.195
(C) Other gastrointestinal disease	38	0.010–0.450	0.100
(D) Cystic fibrosis	7	0.051–0.248	0.219
(E) No gastrointestinal disease disorder	23	0.012–0.300	0.155

Table 2 Serum IgA AGA

	No studied	Optical density units	
		Range	Median
(A) Untreated coeliac disease	15	2–136	45
(B) Coeliac disease on gluten free diet	7	4–11	6
(C) Other gastrointestinal disease	53	1–16	5
(D) Cystic fibrosis	9	1–17	7
(E) No gastrointestinal disease disorder	29	1–49	7

Table 3 Serum IgG AGA

	No studied	Optical density units	
		Range	Median
(A) Untreated coeliac disease	7	1.84–18.40	9.7
(B) Coeliac disease on gluten free diet	3	0.7–5.42	2.36
(C) Other gastrointestinal disease	37	0.32–11.30	1.56
(D) Cystic fibrosis	7	0.3–26.40	0.95
(E) No gastrointestinal disease disorder	16	1.09–14.00	1.4

Serum IgA AGA (fig 2)

As data for both salivary and serum IgA AGA were not available from all patients, the patient

Figure 1 IgA antibodies to crude gliadin in whole unstimulated saliva.

Figure 2 IgA antibodies to gliadin in serum.

numbers are different from those shown in table 1. The results in untreated coeliac disease were significantly higher than in all other groups (fig 2). The 'normal' reference range quoted for the test by Pharmacia is 0–25 and, using this range, three of the 15 patients with untreated coeliac disease had normal values (false negative results), giving the test a sensitivity of 80%. All the patients in the other groups, with one exception, gave results within the quoted normal range, yielding a specificity of 99%.

In the eight patients with coeliac disease in whom saliva and serum AGA were studied simultaneously, there was no correlation between the individual results for salivary and serum IgA. Thus some patients registered high levels of AGA in saliva and low levels in serum, and vice versa.

Serum IgG AGA (fig 3)

Unfortunately, data were available from a relatively small number of patients and, although the median value in the group with untreated coeliac disease was much higher than in the other groups, there was considerable overlap

Figure 3 IgG antibodies to gliadin in serum.

with very high values occurring in children who did not have coeliac disease.

There were no age related differences in values of AGA in saliva or serum in any of the groups (data not shown).

Discussion

The definitive diagnosis of coeliac disease still requires small intestinal biopsy, which is invasive and time consuming, and many negative biopsies are performed each year. Early diagnosis of the condition is important, not only in avoiding the clinical consequences of malabsorption and suboptimal growth, but also because treatment with a gluten free diet appears to reduce the subsequent risk of small intestinal malignancy.²³

In order to minimise the number of negative biopsies, several non-invasive screening tests have been employed and, of these, the use of serological markers has proved to be the most fruitful.²⁴ Thus measurement of serum antibodies to gliadin, reticulin, smooth muscle endomysium, and human jejunum have been employed and various researchers have reported acceptable levels of sensitivity and specificity.^{2–15}

Little is known about mucosal immunity in this condition and the relationship between the antibody concentrations in blood and those in intestinal secretions. Gliadin in the gut lumen is thought to stimulate IgA precursor lymphocytes in the gut associated lymphoid tissue. The precursor lymphocytes differentiate into IgA-producing plasma cells and secrete IgA AGA that enter the intestinal secretions. These precursor lymphocytes also populate mucosal tissues and remote secretory glands such as the salivary glands, resulting in the appearance of IgA AGA in saliva.²⁵ The salivary glands are considered part of the common mucosal immune system although it has been recently suggested that, in adults, salivary antibodies may not reflect intestinal humoral immunity.²⁰ Other data suggest that the measurement of salivary antigliadin antibodies may be a reliable marker for coeliac disease in adult patients²¹ and the aim of our study was to assess their usefulness in childhood.

Our results suggest that measurement of IgA AGA in saliva may be very useful as a screening test for coeliac disease with acceptable levels of sensitivity and specificity. Indeed, using an arbitrary cut off level for our assay in order to obtain 100% sensitivity for coeliac disease, the test remained highly specific. The measurement of serum IgA antibodies using a widely available commercial test kit was less sensitive and, using the kit's quoted reference range, would have missed an unacceptable number of biopsy proved cases of coeliac disease. In the context of screening children for coeliac disease, a salivary IgA AGA test with 100% sensitivity but with 10% false positives is preferable to serum IgA AGA assay with 20% false negative findings. Interestingly, there was poor correlation between salivary and serum IgA AGA in individual patients with untreated coeliac disease and, in our hands, as other workers have suggested, measurement of IgG AGA is too non-specific to

be of clinical usefulness if considered in isolation.¹

The incidence of IgA deficiency is said to be increased in coeliac disease^{4 26} and measurement of IgA antibodies alone may theoretically miss these cases. For this reason measurement of both IgA and IgG antibodies may be more helpful.

Measurement of salivary antibodies is simple, rapid and non-invasive, which would be of great benefit in the investigation of children. Although further studies are needed to confirm the usefulness of salivary antibody measurement and its superiority over serum antibody measurement, a test involving saliva may well prove to be a more acceptable screening tool for coeliac disease before jejunal biopsy than one involving venepuncture.

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- 1 Auricchio S, Greco L, Troncone R. Gluten sensitive enteropathy in childhood. *Pediatr Clin North Am* 1988;35:157-87.
- 2 Stenhammer L, Kilander AF, Nilsson LA, Stromberg L, Tarkowski A. Serum gliadin antibodies for detection and control of childhood coeliac disease. *Acta Paediatr Scand* 1984;73:657-63.
- 3 Lindberg T, Nilsson LA, Borulf S, et al. Serum IgA and IgG gliadin antibodies and small intestinal mucosal damage in children. *J Pediatr Gastroenterol Nutr* 1985;4:917-22.
- 4 Burgin-Wolff A, Berger R, Gaze H, Huber H, Lentze MJ, Nussle D. IgG, IgA and IgE gliadin antibody determinations as screening test for untreated coeliac disease in children: a multicentre study. *Eur J Pediatr* 1989;148:496-502.
- 5 Tucker NT, Barghuthy FS, Prihoda TJ, Kumar V, Lerner A, Lebenthal E. Antigliadin antibodies detected by enzyme-linked immunosorbent assay as a marker of childhood coeliac disease. *J Pediatr* 1988;113:286-9.
- 6 Ascher H, Lanner A, Kristiansson B. A new laboratory kit for anti-gliadin IgA at diagnosis and follow-up of childhood coeliac disease. *J Pediatr Gastroenterol Nutr* 1990;10:443-50.
- 7 Khoshoo V, Bhan MK, Puri S, et al. Serum anti-gliadin antibody profile in childhood protracted diarrhoea due to coeliac disease and other causes in a developing country. *Scand J Gastroenterol* 1989;24:1212-6.
- 8 Calabuig M, Torregosa R, Polo P, et al. Serological markers and coeliac disease: a new diagnostic approach. *J Pediatr Gastroenterol Nutr* 1990;10:435-42.
- 9 Burgin-Wolff A, Gaze H, Hadziselimovic F, et al. Antigliadin and antiendomysium antibody determination for coeliac disease. *Arch Dis Child* 1991;66:941-7.
- 10 Chorzelski TP, Beutner EA, Sulej J, et al. IgA anti-endomysium antibody: a new immunological marker of dermatitis herpetiformis and coeliac disease. *Br J Dermatol* 1984;111:395-402.
- 11 Rossi TM, Kumar V, Lerner A, Heitlinger LA, Tucker N, Fisher J. Relationship of endomysial antibodies to jejunal mucosal pathology: specificity towards both symptomatic and asymptomatic celiacs. *J Pediatr Gastroenterol Nutr* 1988;7:858-63.
- 12 Hallstrom O. Comparison of IgA-class reticulin and endomysium antibodies in coeliac disease and dermatitis herpetiformis. *Gut* 1989;30:1225-32.
- 13 Monteiro E, Livia Menezes M, Magalhaes Ramalho P. Anti-reticulin antibodies: a diagnostic and monitoring test for childhood coeliac disease. *Scand J Gastroenterol* 1986;21:955-7.
- 14 Khoshoo V, Bhan MK, Unsworth JD, Kumar R, Walker-Smith JA. Anti-reticulin antibodies: useful adjunct to histopathology in diagnosing coeliac disease, especially in a developing country. *J Pediatr Gastroenterol Nutr* 1988;7:864-6.
- 15 Kapati S, Burgin-Wolff A, Krieg T, Meurer M, Stolz W, Braun-Falco O. Binding to human jejunum of serum IgA antibody from children with coeliac disease. *Lancet* 1990;336:1335-8.
- 16 Ferguson A, Carswell F. Precipitins to dietary proteins in serum and upper intestinal secretions of coeliac children. *BMJ* 1972;i:75-7.
- 17 Ciclitira PJ, Ellis HJ, Wood GM, Howdle PD, Losowsky MS. Secretion of gliadin antibody by coeliac jejunal mucosal biopsies cultured in vitro. *Clin Exp Immunol* 1986;64:119-24.
- 18 Lycke N, Kilander A, Nilsson LA, Tarkowski A, Werner N. Production of antibodies to gliadin in intestinal mucosa of patients with coeliac disease: study at the single cell level. *Gut* 1989;30:72-7.
- 19 O'Mahony S, Barton JR, Crichton S, Ferguson A. Appraisal of gut lavage in the study of intestinal humoral immunity. *Gut* 1990;31:1341-4.
- 20 O'Mahony S, Arranz E, Barton JR, Ferguson A. Dissociation between systemic and mucosal humoral immune responses in coeliac disease. *Gut* 1991;32:29-35.
- 21 Al-Bayaty HF, Aldred MJ, Walker DM, et al. Salivary and serum antibodies to gliadin in the diagnosis of coeliac disease. *J Oral Pathol Med* 1989;18:578-81.
- 22 Working group of the European Society of Paediatric Gastroenterology and Nutrition. Revised criteria for diagnosis of coeliac disease. *Arch Dis Child* 1990;65:909-11.
- 23 Holmes GKT, Prior P, Lane MR, Pope D, Allan RN. Malignancy in coeliac disease—effect of a gluten-free diet. *Gut* 1989;30:333-8.
- 24 Anonymous. Diagnosis of coeliac disease [Editorial]. *Lancet* 1991;337:590.
- 25 Mestecky J. The common mucosal immune system and current strategies for induction of immune responses in external secretions. *J Clin Immunol* 1987;7:265-76.
- 26 McNicholl B. Coeliac disease: ecology, life-history and management. *Hum Nutr Appl Nutr [Suppl]* 1986;1:55-9.