Screening of diabetic children for coeliac disease with antigliadin antibodies and HLA typing

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Abstract

IgA and IgG antigliadin antibodies were measured in 498 patients with insulin dependent diabetes mellitus and no history of intestinal malabsorption. Thirty patients had abnormal concentrations of antigliadin antibodies; 22 of these had an intestinal biopsy carried out and 16 of the 22 had subtotal villous atrophy suggestive of coeliac disease (prevalence 3·2%). There were no significant differences between patients with coeliac disease and diabetes and diabetic patients with normal IgA antigliadin antibodies in any of the nutritional variables measured, duration of diabetes, and mean insulin requirement. The mean age of onset of diabetes and attainment of expected height for age were both significantly lower in the patients with both diseases. Typing HLA classes I and II was done in 242 patients. The incidence of HLA-B8, DR3, and DQW2, which are commonly associated with both the diseases, is increased when both are present.

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Coeliac disease is more common among patients with insulin dependent diabetes mellitus than among the general population. The estimated incidence among normal children in Europe varies from 1:500 to 1:3000,[1–7] compared with that among diabetic children, which is reported to be between 0·97% and 3·5%[8–15]

Measurement of IgA and IgG antigliadin antibodies in serum using a method with a sensitivity of over 90%,[16–18] has allowed the identification of oligosymptomatic forms (the most common symptoms are low height and weight for age) and asymptomatic forms of coeliac disease.[17 19–22] Screening of children with diabetes for coeliac disease using antigliadin antibodies, has therefore been widely adopted in clinical practice.

Previous studies of genetic[23–25] and immunogenetic aspects,[26–29] and the finding of increased HLA-DR expression on intestinal epithelial cells and pancreatic β cell surfaces in both diabetes and coeliac disease, suggest a common pathogenesis for tissue damage in both diseases.[30 31]

The aims of this study were: to screen children with insulin dependent diabetes for coeliac disease using measurement of antigliadin antibodies, and to identify more detailed HLA associations with both diseases.

Patients and methods

Six centres of paediatric diabetology (Bari, Milan, Naples, Pisa, Turin, and Trieste), which are representative of the Italian paediatric population according to geographical distribution and ethnic background, took part in the study. We studied 498 patients with insulin dependent diabetes mellitus (227 girls, 271 boys; mean age 12·7 years, range 1·1–27·6) at the onset of their diabetes and at follow up visits between August 1987 and April 1988. The mean duration of diabetes was 5·5 years (range 0–20·7) and the mean age at diagnosis was 7·2 years (range 0·3–20·2). None of the patients studied had a history of intestinal malabsorption.

The following clinical and laboratory data were evaluated: age, weight, and height (centile for age), age of onset of diabetes, duration of the illness and insulin requirement, gastrointestinal history, haemoglobin, serum iron and total serum IgA immunoglobulin concentrations (the values were compared with reference ranges for age[32]), one hour xylose test,[33] and IgA and IgG antigliadin antibody concentrations. These assays were carried out in a single centre, using the enzyme linked immunosorbent assay (ELISA) indirect method (double layer).[34] The reference range was calculated on a population of 50 healthy children; the values of the examined samples were calculated as percentages of absorbance with regard to a reference serum pool of positive antigliadin antibodies. Values of antigliadin antibodies more than two standard deviations above the mean were considered abnormal.

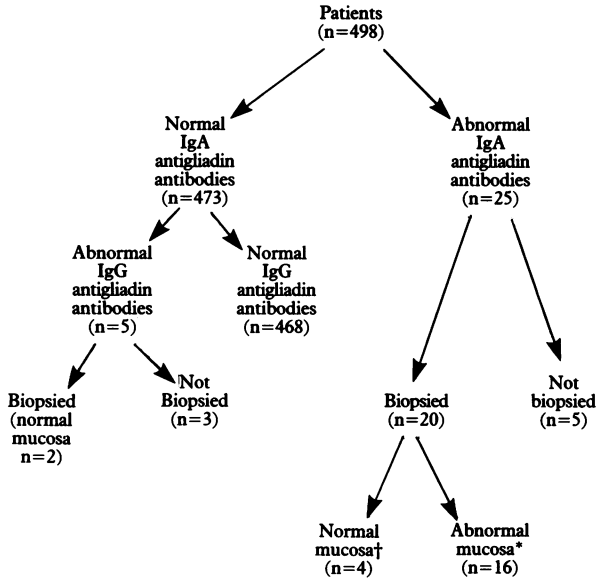
The intestinal biopsy specimens were obtained with a suction capsule and examined histologically.[35] Typing of HLA histocompatibility antigens class I (A, B, and C) and class II (DR-DQ) were carried out at the individual centres in 242 subjects.[36 37] The incidence of the HLA antigens among the subjects studied was compared with that in healthy control groups (800 blood donors from immunohaematology divisions, National Insitute of Cancer, Milan) and with a control group of 26 non-diabetic coeliac patients (mean age 11·1 years, range 1·5–17·9).

The statistical analysis was carried out with Student's t test, the χ^2 test for independent samples, and the χ^2 test with Yates's correction. The p value was multiplied by the number of tested antigens to generate the $p \times n$ value. The data from the various centres were comparable.

Results

Abnormal concentrations of IgA antigliadin antibodies were found in 25 of the 498 patients studied (5%) (table 1). Twenty of these 25

Table 1 Results of the screening tests with IgA and IgG antigliadin antibodies in the 498 patients with diabetes mellitus type 1



*IgG antigliadin antibodies abnormal in nine and normal in seven.
†IgG antigliadin antibodies normal in all.

(80%) underwent an intestinal biopsy, and we found subtotal villous atrophy in 16 subjects (3·2%). Nine of these 16 patients had abnormal concentrations of IgG antigliadin antibodies.

IgG antigliadin antibodies concentrations were abnormal in 14 of the 498 patients (3%); nine also had abnormal concentrations of IgA antigliadin antibodies, associated with subtotal villous atrophy. Two of five patients with abnormal concentrations of IgG antigliadin antibodies and normal IgA antigliadin antibodies underwent intestinal biopsy and the mucosa was histologically normal in both.

The antigliadin antibodies concentrations in patients with mucosal changes reverted to normal after a year on a gluten free diet, and in five subjects who underwent a second biopsy the mucosa had also completely recovered.

Table 2 Comparison of main nutritional variables among patients screened with IgA antigliadin antibodies

	IgA antigliadin antibodies		
	*Abnormal** (n=25)		Normal (n=473)
	Abnormal mucosa (n=16)	Normal mucosa (n=4)	
Abnormal IgG antigliadin antibodies	9	0	5
Abnormal xylose test	4	2	92
Anaemia	0	1	5
Iron deficiency	2	3	44

*Five subjects not biopsied.

Data on xylose tests, iron deficiency and anaemia are given in table 2.

There were no significant differences between patients with coeliac disease and diabetes and diabetic subjects with normal IgA antigliadin antibodies with regard to weight, duration of diabetes, and insulin requirements (table 3). Height, mean age at onset of diabetes and gastrointestinal history were significantly different between the two groups of patients ($p<0·01$).

The incidence of the HLA antigens found in the group of 242 diabetic patients, in the group of patients with diabetes and coeliac disease (n=16), and in the control group (n=800) were compared. Compared with the controls the diabetic subjects had a significantly higher incidence of the antigens HLA-A9, CW7, B8, B18, DR3, DR4, DQW2, and DQW3, and a lower incidence of the antigens HLA-DR2 and DR7. A detailed table is available from the author.

The incidence of the HLA antigens in the diabetic patients with normal concentrations of IgA antigliadin antibodies (n=210) and in those with both coeliac disease and diabetes (n=16) were compared with each other and with a control group made up of 26 patients with coeliac disease. In the patients with both coeliac disease and diabetes the incidence of the HLA A28, B8, B17, and DR3 was significantly higher than in the patients with diabetes alone, while antigen B18 was less common. When compared with the group with coeliac disease alone, the group with both diseases showed an increase in the DR5 antigen and a reduction in the DR7 and DR11 antigens. These differences were, however, not confirmed statistically when the 'p' value was multiplied by the number of tested antigens ($p \times n$).

The comparative evaluation between the non-coeliac diabetic patients and the control group with coeliac disease alone showed the preponderance of the CW4, DR4, and DR5 antigens in the first group, while the coeliac controls had a higher incidence of A24, A26, A30, DR7, DR11, and DQW2 antigens. A detailed table is available from the authors.

Discussion

The results of this multicentre study confirm previous reports (table 4) that there is a higher prevalence of coeliac disease in young patients affected by diabetes mellitus type I (3·2%). This

Table 3 Comparison of auxological and clinical data between diabetic patients with normal IgA antigliadin antibodies and diabetic coeliac patients

	Abnormal IgA antigliadin antibodies, abnormal biopsy specimen (n=16)	*Normal IgA antigliadin antibodies (n=466)*	*p Value*
No (%) with height ≤3rd centile	5 (31)	30 (6)	<0·01
No (%) with weight ≤3rd centile	2 (13)	25 (5)	NS
Mean (range) duration insulin dependent diabetes mellitus: (years)	6·5 (0·9–14·8)	5·4 (0–20·7)	NS
Mean (SD) age at onset of diabetes (years)	4 (2·9)	7·2 (3·6)	<0·01
Mean insulin requirement (U/kg)	0·9	0·83	NS
No (%) gastrointestinal symptoms	9 (56)	48 (10)	<0·01

Table 4 Reported prevalence of coeliac disease in children with insulin dependent diabetes mellitus

First author and reference	*No of patients with insulin dependent diabetes mellitus evaluated*	*No of patients with coeliac disease identified*	*Reported prevalence*
Visakorpi[8]	?	5	1
Thain *et al*[9]	400	6	1·5
Maki *et al*[10]	215	4	2·3
Savilahti *et al*[11]	201	7	3·5
Cacciari *et al*[13]	146	5	3·4
Koletzko *et al*[15]	1032	10–20	0·97–1·94

figure is probably an underestimate, as not all the patients with abnormal values of IgA antigliadin antibodies underwent intestinal biopsy. The assay of serum IgA antigliadin antibodies resulted in a test with greater sensitivity than specificity because of the high number of false positives (four in 20 of the subjects biopsied).

The measurement of IgG antigliadin antibodies seems to be less sensitive, as the test was negative in seven of 16 known coeliac patients (44%). The fact that the intestinal mucosa was normal in the two patients with high concentrations of IgG antigliadin antibodies and normal IgA antigliadin antibodies further emphasises the lack of specificity of the test.

In addition, our results show the unreliability of the one hour xylose test; the number of false negatives was high (62·5% of coeliac patients) as was that of the false positives (19·7%).

We confirmed that in patients with both diabetes and coeliac disease the mean age of onset of the diabetes was significantly lower than in the patients with diabetes alone.[15] The onset of the coeliac disease did not seem to have any connection with the duration of the diabetes. None of the patients tested at the onset of diabetes showed a transitory rise in antigliadin antibodies concentrations, contrary to other reports.[12 13 15]

In patients with coeliac disease and diabetes a high incidence of previous gastrointestinal symptoms was found (56%), although only three of them had any complaints at the time that the coeliac disease was diagnosed. Moreover, subjects with mucosal changes had no significant differences in nutritional variables and in insulin requirements compared with the other diabetic patients.[11 13]

In our experience short stature is the main clinical finding in patients with both coeliac disease and diabetes; although coeliac disease is a well established cause of growth retardation it has not been described previously in patients with both diseases.[17 19–22]

Our data confirm that all diabetic children should be screened for coeliac disease, particularly if they have stunted growth. Of the available screening tests, measurement of antigliadin antibodies is the test of choice.[11–13]

The onset of coeliac disease in diabetic patients does not seem from our study and from previous reports to affect metabolic control of diabetes, and little is known about the onset of nutritional deficits in untreated patients with few or no symptoms.[11 13] The main goal of a gluten free diet in patients with both diseases, therefore, should be the prevention of short stature, even if the final height of patients with coeliac disease is influenced mainly by genetic characteristics.[38] It has been proposed that a strict gluten free diet may lower the increased risk of patients with coeliac disease of developing intestinal malignancies,[39] but this question remains controversial.[40]

The study of the HLA histocompatibility antigens confirms that in our diabetic population, in comparison with the healthy population, antigens B8, DR3 and DR4 are more common, as previously reported, and antigens A9, CW7, DQW2, and DQW3 are also more common, though they are rarely described in association with diabetes. Our evaluation gives further evidence for the protective role of HLA antigens DR2 and DR7 in diabetes.[41 42] The evaluation of HLA antigens in the 16 patients affected by both diabetes and coeliac disease, compared with the control group, confirms the higher incidence of characteristic antigens of both diseases (B8, DR3 and DQW2), with relative risks of 8·4 for the B8, 20 for the DR3, and 7·5 for the DQW2.

The higher incidence of the HLA antigens B8 and DR3 in the group of patients with both coeliac disease and diabetes compared with the non-coeliac diabetic subjects supports a pathogenetic role for an immune response mechanism. These antigens could be in 'linkage disequilibrium' with the immune response genes, and in some way they represent markers of autoimmunity.

To confirm this, these antigens are more common among the group of patients with both diseases; moreover, in these patients the onset of diabetes is significantly earlier ($p<0·01$). This suggests a major autoimmune reactivity in the subjects carrying these HLA antigens. The HLA marker that characterises the association of diabetes and coeliac disease seems to be linked with the antigen DR3.

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Novel experience

The *Drug and Therapeutics Bulletin* is an excellent publication to which we can all look for sound therapeutic advice. Commenting on vigabatrin, however (*Drug and Therapeutics Bulletin* 1990;**28**:95–6), it has come up with an interesting suggestion which could have more general application. 'Treatment should be started', it says, 'by clinicians experienced in its use'. What astute advice about a new drug! 'Were you experienced in the use of this drug, doctor?' 'No, m'lud'. 'Why not?' 'It was new, m'lud'.

The incidence of adverse effects from new drugs can be cut 'at a stroke' by making sure that almost nobody can prescribe them, just as making it illegal to drive a car unless you have driven one before would solve the road traffic problem completely over the next 50 years or so. Or perhaps we could abolish the divorce courts by insisting that only the nuptially experienced should be allowed to marry.

It is sensible to suggest that in the early days most new drugs should be prescribed by doctors who have a large experience of treating the condition for which the drug is to be used. But there's always a first time for all of us.

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