Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2005.02866.x

Anti-endomysial antibody of IgG1 isotype detection strongly increases the prevalence of coeliac disease in patients affected by type I diabetes mellitus

A. Picarelli,*† L. Sabbatella,*†
M. Di Tola,† S. Vetrano,† C. Casale,†
M. C. Anania,† B. Porowska,§
M. Vergari,§ R. Schiaffini¶ and
P. Gargiulo‡

†*Gastroenterology Unit and* ‡*Endocrinology Unit, Department of Clinical Sciences,* §*Department of General Surgery. University of Rome 'La Sapienza', and* ¶*Hospital 'Bambino Gesù', Rome, Italy*

Accepted for publication 12 May 2005
Correspondence: Antonio Picarelli MD, Department of Clinical Sciences, University 'La Sapienza', Viale del Policlinico 155, 00161, Rome, Italy.
E-mail: a.picarelli@flashnet.it

*Both authors contributed equally to this work.

Summary

A strong association between type 1 insulin-dependent diabetes mellitus (IDDM1) and coeliac disease (CD) is well documented, but it is known that prevalence values are underestimated. Serum anti-endomysial antibodies (EMA), considered diagnostic for CD because of their high sensitivity and specificity, belong to the IgA class, but the existence of EMA of IgG1 isotype in the presence or absence of IgA deficiency was reported. In order to re-evaluate the occurrence of CD in IDDM1 patients we performed a screening in IDDM1 patients using EMA of both isotypes. Ninety-four adults affected by IDDM1 (unaffected by CD before enrolling) were enrolled and 83 blood donors as controls. All subjects were on a gluten-containing diet. Histology and biopsy culture were performed. EMA IgA and IgG1 in sera and culture supernatants were detected. Serum EMA were positive in 13 of 94 IDDM1 patients (13·8%). Six of 13 presented IgA-EMA, seven of 13 presented IgG1-EMA. No EMA were found in the control population. Total intestinal atrophy was found in all six patients with serum IgA-EMA and in five of seven with serum IgG1-EMA. Diagnosis of CD was confirmed by histology and organ culture in all 13 patients with serum EMA. The prevalence of CD in the patients affected by IDDM1 was 6·4% for IgA-EMA-positive and 7·4% for IgG1-EMA-positive patients. We confirmed the prevalence of CD in the IDDM1 population obtained with IgA-EMA screening only (6·4%). This prevalence value increases dramatically to 13·8% when IgG1-EMA are also used in the screening. We conclude that IgG1-EMA should also be sought whenever an IDDM1 patient undergoes screening for CD.

Keywords: anti-endomysial antibodies, coeliac disease, IgG1 anti-endomysial antibodies, type I diabetes mellitus, organ culture

Introduction

Coeliac disease (CD) is a permanent intolerance of the small intestine to gluten, characterized by gluten-dependent changes in villous morphology and/or signs of immunological activation detectable in the lamina propria of intestinal mucosa [1–3]. The presence of serum anti-endomysial antibodies (EMA) is generally considered to be highly suggestive for CD because of their high values of sensitivity and specificity [4–6].

The EMAs currently used in the diagnostic work-up of CD are usually of the IgA class only, but recent studies have reported the existence of a new class of CD subjects presenting with EMA of IgG1 isotype in the presence as well as the absence of IgA deficiency [7–9]. The presence of IgG1 EMA causes relevant changes in the prevalence of this illness, actually estimated to be higher than that reported (1 : 180) [10,11].

In the literature a strong association between type-I insulin dependent diabetes mellitus (IDDM1) and CD is well documented [12]. It is well known that the prevalence of CD in IDDM1 patients is higher than that of the healthy population [13], and can be up to 20 times higher [14]. In addition, it has recently been noted that a subset of IDDM1 children showed an abnormal response of the intestinal mucosa to gluten [15].

In recent years several studies have been performed to address the incidence of CD in IDDM1 patients, showing

that CD is occurring commonly in IDDM1 patients [16], with a prevalence ranging between 2% and 8% depending on the screening methods used [17–19]. However, it is a well-recognized fact that the association between these two diseases is underestimated [19].

In addition it has been reported that IDDM1 patients, particularly adults, affected by CD present in atypical or oligosymptomatic form [20,21], as has been observed in CD patients with IgG1 EMA-positive [9].

Moreover, it has been reported previously that the detection of IgG1 EMA in patients who are affected by IDDM1 could increase the prevalence of CD in these patients, allowing CD to be diagnosed in patients which otherwise might not be detected [22].

In light of this evidence we performed a screening in a population of patients affected by IDDM1 using anti-endomysial antibodies not only of IgA isotype, but also of IgG1 isotype aiming to re-evaluate the occurrence of CD in IDDM1 patients and also to evaluate if using IgG1 EMA that the prevalence of CD in IDDM1 patients would increase in the same way as it has in the general population.

Materials and methods

Subjects

Ninety-four consecutive adults patients affected by IDDM1 (43 males, 51 females, mean age 46·9 years, range 18–70 years) all regularly attending our Center for the Study of Diabetes (for a minimum of 5 years) were enrolled into this study. None of these patients presented any symptoms attributable to an enteropathy and any evidence of malabsorption from other laboratory variables, and none had been diagnosed previously with coeliac disease before enrolling in the study.

All anamnestic, clinical and metabolic data of these patients are reported in Table 1. Patients were selected randomly by the same diabetologist. All patients were treated with subcutaneus human insulin (regular and long-acting). None of the patients received insulin by infusion pump.

Eighty-three (38 males and 45 females, mean age 32·3 years, range 20–52 years) blood donors were also enrolled as healthy controls. None of these subjects was affected by IDDM1, CD or other autoimmune diseases or had a first-degree relative who was affected by one of these diseases. All subjects enrolled in this study were on a gluten-containing diet.

Informed consent was obtained from all patients and all the procedures followed were in accord with the ethical standards of the Institutional Committee responsible for human experimentation.

Table 1. Details of the subjects under study.

	Mean ± s.d.
Patients (no.)	94
Sex (M/F)	43/51
Age (years)	46·9 ± 10·3
Duration of diabetes (years)	16·4 ± 9·4
Glycosylated haemoglobin (%)	7·06 ± 1·30
Total cholesterol (mg/dl)	208·5 ± 56·4
HDL cholesterol (mg/dl)	45·2 ± 8·2
Triglycerides (mg/dl)	149·2 ± 46·2

Collection and processing of blood samples

Fasting blood samples were collected with minimal venous stasis using a 19-gauge 'butterfly' needle and polypropylene syringes preloaded with the appropriate solutions.

To determine glycosylated haemoglobin (HbA1c), 3 ml of blood was mixed with ethylene diamine tetra-acetic acid (EDTA) and stored at 4°C; HbA1c was determined spectrophotometrically by using commercially available reagents (Bio-Rad, Richmond, CA, USA). The normal range in our laboratory is 4·0–6·0%.

To determine serum glucose and lipids (total cholesterol, HDL cholesterol and triglycerids), 5 ml of serum was processed by routine autoanalyser methodology with enzymatic techniques (Boehringer Mannheim, Germany).

Duodenal biopsy

Three biopsy specimens of duodenal mucosa were obtained for diagnostic purposes from all the patients with sera EMA IgA- and IgG1-positive results.

One specimen was submitted for routine histological examination by means of haematoxylin–eosin staining. The degree of intestinal atrophy and crypt hyperplasia were evaluated and the results compatible with class III (a, b and/or c) of the Marsh classification, as modified by Oberhuber *et al.* [23] were considered pathognomonic of coeliac disease. The other two samples were submitted to organ culture.

Biopsy culture

Two biopsy samples were cultured for 48 h at 37°C, one in the presence and one in the absence of peptic-tryptic (PT) digest of gliadin (1 g/l), using the 'in batch' method suggested recently [24].

Culture supernatants were collected and stored at −70°C until they were used for both IgA and IgG1 EMA detection.

Anti-endomysial antibody detection

EMA either of IgA or IgG1 isotype were sought in sera diluted 1 : 5 from all subjects under observation, using a commercially available kit (Eurospital, Trieste, Italy) on cryostat sections of monkey oesophagus. Positive EMA results were identified by the typical reticulin-like staining of smooth muscle bundles.

Table 2. Serum EMA-positive results in the populations studied.

Subjects	Serum IgA EMA	Serum IgG1 EMA
IDDM1 patients ($n = 94$)	6/94 (6·4%)	7/94 (7·4%)
Healthy controls ($n = 83$)	0/83	0/83

EMA both IgA and IgG1 were also sought in undiluted culture supernatants obtained from patients with a positive sera IgA and/or IgG1 EMA results.

The results were evaluated blindly by two observers. The agreement rate was 98·8%.

Detection of total IgA

Total IgA immunoglobulins were measured by a radial immunodiffusion method (Easy Rid IgA, Liofilchem Bacteriology Products, Teramo, Italy). Results were evaluated by reference to a standard curve; normal values in adult patients ranged between 90 and 450 mg/dl. According to the manufacturer's instructions, 90 mg/dl was used as cut-off value to identify IgA deficiency.

Statistical analysis

Data are expressed as means ± standard deviation (s.d.). Statistical analysis was performed using the correlation between variables by Student's unpaired *t*-test.

Results

All diabetic patients included in the study were adult IDDM1 with a long duration of the disease (>15 years) and satisfactory metabolic control (Table 1).

Weight flux and body mass index values were been monitored during 1 year of follow-up and no significant differences were observed for both parameter either in patients with serum EMA-positive or EMA-negative.

Anti-endomysial antibodies were positive in the sera of 13 of the 94 IDDM1 patients screened (13·8%). Six of these 13 patients presented with EMA of IgA isotype, while the other seven were IgG1 EMA-positive. No EMA were detectable in sera from the healthy subjects belonging to the control population (Table 2).

All the patients studied presented normal levels of total serum IgA, irrespective of the presence of EMA and their isotype, therefore none of them had an IgA deficiency.

All six patients with serum IgA EMA-positive presented a total or subtotal intestinal mucosa atrophy (types IIIb and IIIc of the Marsh classification, as modified by Oberhuber *et al.*) [23]. Total or subtotal intestinal mucosa atrophy (types IIIb and IIIc of the Marsh classification, as modified by Oberhuber *et al.*) [23], was also found in five of the seven patients with serum IgG1 EMA-positive. The remaining two patients presented an intestinal mucosa architecture compatible with type II of the Marsh classification, as modified by Oberhuber *et al.* [23] (Table 3).

All six patients with serum IgA EMA-positive were also IgA EMA-positive in culture supernatants, irrespective of the presence or absence of PT-gliadin. In the same way, all seven IgG1 EMA-positive patients presented with IgG1 EMA-positive in culture supernatants irrespective of the presence or absence of PT-gliadin (Table 3).

Therefore, the diagnosis of coeliac disease was confirmed by histology and organ culture in all the 13 patients presenting with serum EMA-positive. The prevalence of CD in the population of patients affected by IDDM1 studied was 6·4% for IgA EMA-positive patients and 7·4% for IgG1 EMA-positive patients, respectively.

The mean age and the duration of disease of IDDM1–EMA-positive patients is similar to that of IDDM1–EMA-negative patients, but the metabolic control was significantly different between the two groups: the IDDM1 patients with EMA-positive results presented significantly lower concentrations of glycolipid parameters (Table 4).

Discussion

Coeliac disease, a life-long gluten intolerance of the small intestine, occurs commonly in patients affected by IDDM1 [12–14]. It is well known that patients already affected by IDDM1 are at a higher risk of developing CD compared with the normal population [13,14].

Despite this observation it has been reported that CD is an underestimated disease, both in the normal population and in patients already affected by IDDM1 [18].

Moreover, recent work has shown the presence of a condition of abnormal mucosal immune response to gluten in a subset of IDDM1 children not affected by CD [15]. This observation allows us to suppose the existence of an abnormal basal hypersensitivity to gluten in patients already affected by IDDM1.

The strong association between these two diseases could be explained on the basis of their shared autoimmune

Table 3. Histological and culture EMA-positive results in serum EMA-positive patients.

Subjects	Intestinal atrophy*	Culture supernatants IgA EMA	Culture supernatants IgG1 EMA
Serum IgA-positive patients ($n = 6$)	6/6	6/6	0/6
Serum IgG1-positive patients ($n = 7$)	5/7	0/7	7/7

*Type III of the Marsh classification as modified by Oberhuber *et al.* [22].

Table 4. Metabolic variables in diabetic patients in relation to the presence of EMA.

	EMA-positive	EMA-negative	*P*
Patients (no.)	13	81	
Age (years)	44·9 ± 13·6	50·8 ± 10·3	n.s.*
Duration of diabetes (years)	19·7 ± 11·4	17·8 ± 7·9	n.s.*
Glycosylated haemoglobin (%)	6·49 ± 0·8	7·40 ± 1·3	<0·05
Cholesterol (mg/dl)	178·0 ± 25·8	239·5 ± 35·7	<0·001
Triglycerides (mg/dl)	96·0 ± 30·7	156·0 ± 25·8	<0·001

*n.s.: not significant.

nature, on the existence of a common genetic background, the same molecule HLA-DQ2, as well as on that of a common pathogenetic mechanism [25,26]. Because of the high association between these two diseases, some authors have proposed carrying out screening for CD in all newly diagnosed IDDM1 patients [27].

Furthermore, it is known that CD patients present a higher risk of developing other autoimmune diseases [19] and it seems that organ-specific autoantibodies, especially thyroid-related and diabetes-related, can be gluten-dependent and that they can disappear during a gluten-free diet (GFD) [28]. Taking this into account, a GFD started early could prevent the development of IDDM1 in genetically predisposed CD patients [25,29,30], but this observation is still being debated [31].

It is worth pointing out that in our clinical practice none of the CD patients during a gluten-free diet developed IDDM1. Moreover, the observation of a low concentration of metabolic parameters in EMA-positive IDDM1 patients seems to be related to the abnormal intestinal absorption that characterizes coeliac disease. This observation could suggest a possible protective role of CD in the development of vascular diabetic complications.

The presence of anti-endomysial antibodies of IgA isotype in sera of untreated CD patients and their disappearance after a strictly maintained gluten-free diet, together with their high sensitivity and specificity, justify the use of EMA as a standing tool for the screening of CD [4–6]. Despite the above, EMA sensitivity has been further improved recently by the discovery of EMA of IgG1 isotype in patients affected by CD but serum IgA-negative, in the presence as well as the absence of IgA deficiency [7–9].

In the present study we have confirmed the prevalence of CD in IDDM1 patients obtained when only IgA EMA are used in the screening (6·4%) and in addition we have shown that this value improves dramatically to 13·8% when IgG1 EMA are also used in the screening.

In accordance with data obtained by other authors who showed that the prevalence of CD in IDDM1 children is higher than that of the normal population, together with the observation that most of the CD patients diagnosed at the onset of IDDM1 did not present any symptom [14] and with our results, we suggest that patients who undergo a diagnosis of IDDM1 should undergo screening for anti-endomysial antibodies, not only of IgA but also of IgG1 isotype.

Acknowledgements

This study was supported partially by the Ministry of University and Research (MIUR) research project 'Alimentazione e celiachia (ALICE): tossicità, immunogenicità e sicurezza d'uso alimentare di cereali e specie affini'. The study was also supported partially by the non-governmental association for research on coeliac disease and diabetes mellitus 'CELIDIA 2001'.

References

1 Ciclitira PJ. Recent advances in coeliac disease. Clin Med 2003; **3**:166–9.
2 Fasano A, Catassi C. Current approaches to diagnosis and treatment of coeliac disease: an evolving spectrum. Gastroenterology 2001; **120**:636–51.
3 Picarelli A, Maiuri L, Mazzilli MC *et al.* Gluten-sensitive disease with mild enteropathy. Gastroenterology 1996; **111**:608–16.
4 Ferreira M, Lloyd Davies S, Butler M, Scott D, Clark M, Kumar P. Endomysial antibody: is it the best screening test for coeliac disease? Gut 1992; **33**:1633–7.
5 Corrao G, Corazza GR, Andreani ML *et al.* Serological screening of coeliac disease: choosing the optimal procedure according to various prevalence values. Gut 1994; **35**:771–5.
6 Bottaro G, Volta U, Spina M, Rotolo N, Sciacca A, Musumeci S. Antibody pattern in childhood coeliac disease. J Pediatr Gastroenterol Nutr 1997; **24**:559–62.
7 Cataldo F, Lio D, Marino V, Picarelli A, Ventura A, Corazza GR. IgG (1) antiendomysium and IgG antitissue transglutaminase (anti-tTG) antibodies in coeliac patients with selective IgA deficiency. Working Groups on Coeliac Disease of SIGEP and Club del Tenue. Gut 2001; **47**:366–9.
8 Picarelli A, Sabbatella L, Di Tola M *et al.* Coeliac disease diagnosis in misdiagnosed children. Pediatr Res 2000; **48**:590–2.
9 Picarelli A, Di Tola M, Sabbatella L *et al.* Identification of a new coeliac disease subgroup: antiendomysial and anti-transglutaminase antibodies of IgG class in the absence of selective IgA deficiency. J Intern Med 2001; **249**:181–8.
10 Catassi C, Ratsch IM, Fabiani E *et al.* Coeliac disease in the year 2000: exploring the iceberg. Lancet 1994; **343**:200–3.
11 Fasano A, Berti I, Gerarduzzi T *et al.* Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med 2003; **163**:286–92.

12 Savilahti E, Simell O, Koskimies S, Rilva A, Akerblom H. Coeliac disease in insulin-dependent diabetes mellitus. J Pediatr 1986; **108**:690–3.
13 Cronin CC, Feighery A, Ferriss JB, Liddy C, Shanahan F, Feighery C. High prevalence of coeliac disease among patients with insulin-dependent (type I) diabetes mellitus. Am J Gastroenterol 1997; **92**:2210–2.
14 Barera G, Bonfanti R, Viscardi M *et al.* Occurrence of coeliac disease after onset of type 1 diabetes: a 6 year prospective longitudinal study. Pediatrics 2002; **109**:833–8.
15 Troncone R, Franzese A, Mazzarella G *et al.* Gluten sensitivity in a subset of children with insulin dependent diabetes mellitus. Am J Gastroenterol 2003; **98**:590–5.
16 Holmes GK. Coeliac disease and type I diabetes mellitus – the case for screening. Diabet Med 2001; **18**:169–77.
17 Barera G, Bianchi C, Calisti L *et al.* Screening of diabetic children for coeliac disease with antigliadin antibodies and HLA typing. Arch Dis Child 1991; **66**:491–4.
18 Cronin C, Shanahan F. Insulin dependent diabetes mellitus and coeliac disease. Lancet 1997; **349**:1096–7.
19 Not T, Tommasini A, Tonini G *et al.* Undiagnosed coeliac disease and risk of autoimmune disorders in subjects with type I diabetes mellitus. Diabetologia 2001; **44**:151–5.
20 Sjoberg K, Eriksson KF, Bredberg A, Wassmuth R, Eriksson S. Screening for coeliac disease in adult insulin-dependent diabetes mellitus. J Intern Med 1998; **243**:133–40.
21 Talal AH, Murray JA, Goeken JA, Sivitz WI. Coeliac disease in an adult population with insulin-dependent diabetes mellitus: use of endomysial antibody testing. Am J Gastroenterol 1997; **92**:1280–4.
22 Sabbatella L, Di Tola M, Picarelli A. The high frequency of coeliac disease and other autoimmune diseases in subjects affected by type I (insulin-dependent) diabetes mellitus and in their first degree relatives. Diabetologia 2002; **45**:748.
23 Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999; **11**:1185–94.
24 Picarelli A, Sabbatella L, Di Tola M *et al.* Forty-eight hours of biopsy culture improve the sensitivity of the *in vitro* gliadin challenge in the diagnosis of coeliac disease. Clin Chem 2001; **47**:1841–3.
25 Pocecco M, Ventura A. Coeliac disease and insulin dependent diabetes mellitus – a causal association? Acta Pediatr 1995; **84**:1432–3.
26 King AL, Moodie SJ, Fraser JS *et al.* Coeliac disease: investigation of proposed casual variants in the CTLA4 gene region. Eur J Immunogenet 2003; **30**:427–32.
27 Carlsson AK, Axelsson IE, Broulf SK *et al.* Prevalence of IgA-antiendomysium and IgA-antigliadin autoantibodies at diagnosis of insulin-dependent diabetes mellitus in Swedish children and adolescents. Pediatrics 1999; **103**:1248–52.
28 Ventura A, Neri E, Ughi C, Leopoldi A, Citta A, Not T. Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with coeliac disease. J Pediatr 2000; **137**:263–5.
29 Lorini R, Scotta MS, Avanzini MA, Vitali L. IgA antibodies to gliadin, reticulin and endomysium for coeliac disease screening in children with insulin-dependent diabetes mellitus. J Pediatr 1994; **124**:994–6.
30 Ventura A, Magazzù G, Greco L. Duration of exposure to gluten and risk for autoimmune disorders in patients with coeliac disease. Gastroenterology 1996; **117**:297–303.
31 Schuppan D, Hahn EG. Coeliac disease and its link to type I diabetes mellitus. J Pediatr Endocrinol Metab 2001; **14** (Suppl. 1):597–605.