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Prevalence of silent celiac disease in patients with autoimmune thyroiditis from Northern Sardinia

G.F. Meloni*, P.A. Tomasi**, A. Bertoncelli**, G. Fanciulli**, G. Delitala**, and T. Meloni*

*Pediatric Clinic "A. Filia", and **Institute of Special Medical Pathology, University of Sassari, Sassari, Italy

ABSTRACT. Celiac disease (CD) is frequently associated with other autoimmune diseases such as Type 1 diabetes mellitus, autoimmune thyroiditis (AT), and Addison's disease. The frequency of these associations varies with the populations studied. We conducted this study to ascertain the prevalence of CD in patients with AT from Sardinia, an area with a very high prevalence of CD. To this aim, 297 consecutive patients with AT (as defined by elevated antithyroid antibody levels and a positive ultrasound scan) were studied. Immunoglobulin A and G-class antigliadin antibodies were assayed in serum; if either or both were positive, antiendomysium antibodies were determined. If two markers were positive, serum ferritin, folate, and vitamin B12 levels were measured and jejunal biopsy was suggested. Thirteen out of the 14 patients who showed at least two positive markers consented to jejunal biopsy and all of them showed histological features of CD. The prevalence of CD in AT patients was 4-fold greater than that observed in the general population (4.37 *vs* 1.06%, $p<0.0001$). Ferritin was low in 6 and vitamin B12 in 2 out of 13 patients; serum folates were normal in all patients. Molecular typing of HLA class II alleles showed an increased frequency of the extended haplotype DRB1*0301/DQA1*0501/DQB1*0201. None of our patients had a history of gastrointestinal symptoms. We confirm the increased prevalence of silent CD in patients with AT. Patients with AT ought to be regarded as a high-risk group for CD and should be screened routinely for it; if negative, screening tests should be repeated at regular intervals.

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INTRODUCTION

Celiac disease (CD) is a permanent intolerance to gliadin responsible for an immunologically mediated small intestinal enteropathy in genetically susceptible individuals (1). The clinical presentation of CD is extremely heterogeneous and ranges from its "classical" form, characterized by overt gastrointestinal symptoms, to oligosymptomatic or even asymptomatic forms presenting with mild and often misleading clinical manifestations not directly related with the gastrointestinal tract or with no symptoms at all (1). Another recognized feature of CD is its close association with other autoimmune diseases, such as Type 1 diabetes, with which CD probably shares a common genetically determined predisposing asset (1-3).

Key-words: Thyroiditis, celiac disease, antigliadin antibodies, antiendomysium antibodies, HLA alleles, hypothyroidism, hyperthyroidism.

Correspondence: Dr. G.F. Meloni, Clinica Pediatrica, Viale S. Pietro 12, 07100, Sassari, Italy.

E-mail: gfmeloni@pharm.medicina.uniss.it

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Recent research showed an increased prevalence of autoimmune thyroiditis (AT) in patients with CD (4-8). The availability of non-invasive and relatively simple serological screening tests, such as the measurement of serum antigliadin (AGA), antireticulin or antiendomysium antibodies (AEA) (9-11) allowed to confirm this association by detecting CD in patients with AT (12-14).

Sardinia is a region with a high prevalence of thyroid disease; previous studies showed a high prevalence of positive antithyroperoxidase (anti-TPO) antibodies in the general population (13.7%) (15), and of goiter in a juvenile cohort (21 to 61%) (16).

We undertook the present study in order to confirm this association in Northern Sardinia, where the highest prevalence ever of CD has been described to date (17), by screening a substantial number of patients with serologically identified TA.

MATERIALS AND METHODS

We studied 297 patients with AT, who were followed by the Section of Endocrinology and the Department of Pediatrics at the University of Sassari. We selected

all the patients with thyroiditis who were seen in the year 1996 (no.=301); 4 patients were excluded because of missing data.

The age range of our subjects was 12 to 79 yr (mean±SD, 46.6±14 yr), with 271 female (91.2%) and 26 male patients (8.8%).

The diagnosis of AT was made on clinical and biochemical grounds on the basis of positive titres of anti-TPO and/or anti-thyroglobulin (anti-TG) antibodies, and a positive ultrasound scan (as defined by either a diffuse marked hypoechogenicity or a focal non-homogeneous pattern with hypoechogeneous areas).

We included asymptomatic patients, and patients who were under levothyroxine treatment for hypothyroidism, 32 out of the 297 patients were hypothyroid (as shown by high TSH values) and 33 had increased free T_4 values.

One of the 297 patients included in the study also had Addison's disease, and another had Type 1 diabetes mellitus.

Venous blood samples were drawn from all the subjects enrolled in the study. Following separation by centrifugation, the sera were stored at -80 C.

TSH values were determined with a commercial non-competitive immunometric luminescence assay (TSH Vitros – Ortho Clinical Diagnostics, Amersham, UK); the lower and upper reference limits were 0.46 and 4.68 mU/ml respectively; precision data of the assay are as follows: maximum coefficient of variation=4.7% (intracalibration at a level of 39 mU/l). Anti-TG and anti-TPO antibodies were assayed using commercial IRMA kits (Anti HTG bridge, Biochem Immunosystems, Italy; and AB-TPOK-3, DiaSorin, Saluggia, Italy), with the upper reference limits being 200 U/ml and 12 arbitrary U/l (AU/l), respectively. Intra-assay coefficients of variation for the antibody assays at low, medium and high level are, respectively: 5.0%, 3.6% and 9.9% for anti-TG and 4.1%, 6.1% and 2.4% for anti-TPO; the inter-assay values are 6.3%, 9.2% and 8.3% for anti-TG, and 9.1%, 4.8% and 6.5% for anti-TPO.

The AGA assays, for both IgA- and IgG (immunoglobulin A and G)-class, were carried out by ELISA (Alfa-Gliatest, Eurospital, Trieste, Italy). Cut-off values of 7 AU for AGA IgA and of 15 AU for AGA IgG, respectively, were established by the manufacturer, based on the screening of a large number of both CD patients and healthy controls.

All sera that tested positive for IgA- and/or IgG-class AGA were subsequently tested for IgA-class AEA by indirect immunofluorescence, using sections of human umbilical cord which were then counter-stained with fluorescein-labelled goat antihuman IgA (Endomisio HUC, Eurospital, Trieste, Italy). Since selective IgA deficiency is present in 1:40-70 of patients with CD (1, 2), total serum IgA was measured in all the sera found positive only for IgG-class AGA, in order to exclude a selective IgA deficiency. In all subjects who tested positive for at least 2 of the 3 markers (IgA- and IgG-class AGA, AEA) serum ferritin, folates and vitamin B12 levels were assayed, and the patients were then asked to consent to a jejunal biopsy. Biopsy specimens were subsequently taken from the descending part of the duodenum by upper intestinal endoscopy in all the patients that agreed to the procedure. All specimens were examined by the same pathologist and classified according to the criteria proposed by Marsh (18). Molecular typing of HLA class II DRB1, DQA1 and DQB1 alleles was determined by the polymerase chain reaction - sequence specific primers (PCR-SSP) method, as previously described by other authors (19-21). Putative HLA class II haplotypes were established on the basis of known linkage disequilibrium in Caucasians (22, 23).

As a control group for the determination of the prevalence of CD in the general population of Northern Sardinia we used the data obtained from a previous screening study for CD carried out on a sample of 1607 apparently healthy school-children from Northern Sardinia that showed a 1.06% (no.=17) prevalence of histologically proven CD (17).

Prevalence rates of silent CD in the two groups were compared by the χ^2 test.

RESULTS

Ten subjects (3.4%) were positive for IgG-class AGA but not for IgA-class AGA. Of these, 8 were AEA-negative and were therefore excluded from further study; none of the 8 subjects had a selective IgA deficiency. The remaining 2 subjects (positive for IgG-class AGA and AEA, but IgA-class AGA-negative) were included in further analysis.

Five patients (1.7%) were positive for IgA-class AGA but not IgG-class AGA. Of these, 4 were also AEA-positive and were thus included in further analysis.

Eight patients (2.7%) were positive for both IgG- and IgA-class AGA; all were also AEA-positive.

In total, 14 patients were positive for at least 2 of the 3 markers and were therefore considered eligible for jejunal biopsy; one of them (who was positive for IgA-class AGA and AEA and negative for IgG-class AGA) however denied consent to the procedure. Biopsy samples from all these 13 subjects showed marked mucosal flattening with severe villous atrophy and crypt hyperplasia (10 scored 3 and 3 scored 2).

The prevalence of histologically confirmed CD in our patients was thus 4.37% (13/297); none of these patients complained of any overt symptoms attributable to the disease.

Table 1 - *Laboratory and histological findings of the 13 patients with autoimmune thyreopathy and celiac disease.*

Patient	Sex	Age (yr)	Duration of autoimmune thyroiditis (yr)	Serum ferritin	Serum folates	Serum vitamin B12	IgA AGA normal	IgG AGA	AEA	Jejunal biopsy CD grade
1	F	22	3	8.54	3.3	491	8.44	10.37	Positive	3
2	F	63	8	4.77	5.9	450	19.38	62.46	Positive	3
3	F	65	4	143.75	10.5	610	1.54	25.04	Positive	3
4	F	48	5	3.09	6.0	556	16.01	8.02	Positive	2
5	F	65	4	11.99	15.9	140	22.56	4.41	Positive	3
6	F	42	1	7.10	9.2	714	11.76	48.6	Positive	2
7	M	43	4	31.24	4.3	810	13.41	24.77	Positive	3
8	F	44	3	4.34	3.0	322	5.21	44.01	Positive	3
9	F	61	2	52.05	7.0	417	14.20	44.80	Positive	3
10	F	15	1	22.78	7.2	537	29.83	94.76	Positive	3
11	F	18	1	14.55	7.7	112	10.16	27.10	Positive	3
12	F	12	1	31.12	4.1	1222	23.93	94.76	Positive	2
13	F	45	1	6.85	3.4	400	13.13	24.02	Positive	3

Serum ferritin normal range: males 22-322 µg/ml, females 10-291 µg/ml; serum folates normal range: 1.1-20 µg/ml; serum vitamin B12 normal range: 211-911 µg/ml; IgA AGA normal range: 0-7 U; IgG AGA normal range: 0-15 U. AEA: antiendomysium antibodies; AGA: serum antigliadin; CD: celiac disease; F: female; M: male.

Serum folates were within normal limits in all 13 patients, whereas 6 out of 13 (46.2%) patients had low ferritin values and 2 (15.4%) had low vitamin B12 levels.

Molecular typing of HLA class II alleles showed a marked increase of the heterodimer DQA1*0501/DQB1*0201. In fact, 8 out of 9 subjects whose DNA was available, carried the genes coding for the DRB1*0301/DQA1*0501/DQB1*0201 extended haplotype (Table 2), and another one possessed a DRB1*0405 allele which is also known to be associated with CD (21). A double dosage of genetic susceptibility to CD was detected in 5 of the 9 CD patients (3 of them were homozygous for the DRB1*0301/DQA1*0501/DQB1*0201 extended haplotype and 2 of them carried one copy of the same haplotype in association to a copy of the DRB1*0405 allele and a copy of the DQA1*0101/DQB1*0501 heterodimer respectively).

The prevalence of subclinical CD in patients with AT was significantly higher from that observed in the general population of the same geographical area: 13 out of 297 *vs* 17 out of 1607 ($p<0.0001$).

Table 2 - *Distribution of DRB1, DQA1 and DQB1 genotypes in 9 patients with autoimmune thyroiditis and celiac disease.*

Patient	DRB1*	DQA1*	DQB1*	DRB1*	DQA1*	DQB1*
1	0301	0501	0201	0301	0501	0201
2	0301	0501	0201	1302	0102	0609
3	0301	0501	0201	1401	0104	0503
4	0301	0501	0201	0405	0501	0301
6	0102	0101	0501	0301	0501	0201
7§	0301	0501	0201	0403	0303	0301
8	0301	0501	0201	0301	0501	0201
9	0301	0501	0201	0301	0501	0201
12°	0405	0303	0302	0804	0401	0402

§Addison's disease; °Type 1 diabetes mellitus.

DISCUSSION

The association between CD and autoimmune diseases, including thyroiditis (4-9, 12-14), is not surprising since these conditions share common HLA haplotypes (1, 6, 7, 25, 26). As a rule, in patients with Type 1 diabetes mellitus, CD is diagnosed after the onset of diabetes and is usually asymptomatic (27). The same phenomenon might indeed also exist in patients with AT (5, 6). On the other hand, since our patients all suffered from silent CD and were therefore undiagnosed before this study, it is also possible that a prolonged exposure to gluten might have triggered the development of another autoimmune condition; in children, the risk of developing an autoimmune disorder in CD ap-

pears, according to some authors (28), to be correlated to the duration of the gluten-containing diet. Our data are in agreement with those of Collin *et al.* (12) and show a high prevalence (4.37%) of CD in patients with AT; this prevalence might have been even higher (4.82%) if our 14 patients had consented to a jejunal biopsy. CD in our patients with AT is approximately 4 times more frequent than in the general population of the same geographical area (4.37% *vs* 1.06%) in agreement with other data obtained in Italy (14).

One out of the 13 patients with AT and CD, also had Addison's disease (patient 7) and another one had Type 1 diabetes mellitus (patient 12). Similar associations have been reported before (3, 4, 29) and it could be hypothesized that CD is possibly to be included in the manifestations of polyglandular autoimmune endocrine failure.

Iron deficiency due to depletion of iron stores is, in our experience, one of the most common clinical presentations of atypical CD. In this study we observed a high prevalence of iron deficiency also in patients with AT and silent CD; 46% of our patients with AT and CD had low ferritin values. This percentage is similar to those observed by our group in apparently healthy school-age children with silent CD (17) diagnosed during a screening study and also to those observed in children or young adults with Type 1 diabetes mellitus and CD (Meloni G.F. *et al.*, unpublished data).

Molecular typing of HLA class II alleles showed a homogeneous genetic predisposing background. In fact, 8 subjects (66.6%) carried the genes coding for the DRB1*0301/DQA1*0501/DQB1*0201 extended haplotype (Table 2). A double dosage of genetic susceptibility to CD was detected in 5 of the 9 CD patients, 3 of whom were homozygous for this particular haplotype, 2 of whom carried one copy of the same haplotype in association to a copy of the DRB1*0405 allele and one copy of the same haplotype in association with a DQA1*0101/ DQB1*0501 heterodimer respectively. The high prevalence (23%) of the DRB1*0301/DQA1*0501/ DQB1*0201 extended haplotype among Sardinians has been reported before (24) and its frequency is probably ascribable to a phenomenon of genetic drift due to the insularity of Sardinia and the high level of endogamy induced by difficult internal communications.

Our data suggest that patients with newly-diagnosed AT should be screened for CD, particularly young patients and those patients with another endocrine autoimmune disorder. The implementation of such a screening could help to identify whether the presence of gluten-sensitive enteropathy precedes the onset of AT or follows it (as it appears to be the case in Type 1 diabetes mellitus) (27).

If the initial screening is negative, AGA and AEA assays should be repeated at regular intervals to allow early identification of CD. In fact, untreated CD has been associated with an increased incidence of intestinal lymphomas (30, 31) and the treatment with a gluten-free diet might prevent this complication. Moreover, the recovered mucosal integrity probably allows a more efficient replacement therapy in hypothyroid patients. Further studies aimed to detect the association of different diseases that share a common autoimmune basis are needed to shed some light on the underlying mechanisms that ultimately lead to organ specific manifestations.

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