CLINICAL STUDY

Celiac disease in North Italian patients with autoimmune Addison’s disease

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Abstract

Objective: Patients with autoimmune Addison’s disease (AAD) are prone to develop other autoimmune manifestations. An increased prevalence of celiac disease (CD) has recently been demonstrated in Northern European patients with AAD. IgA deficiency is the most frequent type of immunodeficiency among humans and is present in about one in every 600 individuals in the population. IgA deficiency is frequent in patients with other autoimmune diseases, but data concerning AAD are still unavailable.

Design: The aim was to define the prevalence of CD and of IgA deficiency in a group of Italian patients with AAD.

Methods: One hundred and nine patients with AAD were enrolled and examined for tissue transglutaminase autoantibodies of the IgA class, circulating levels of IgA and adrenal cortex antibodies.

Results: Two (1.8%) of the patients were affected by already diagnosed CD and were already on a gluten-free diet. Out of the remaining 107 patients, four (3.7%) were found to be positive for IgA antibodies to human tissue transglutaminase. Three of the four patients who were positive for tissue transglutaminase autoantibodies agreed to undergo endoscopy and duodenal biopsies and, in one, a latent form of CD was identified. The clinical, silent or latent form of CD was present in six out of 109 (5.4%). This prevalence was significantly higher \( P = 0.0001 \) than that reported for the Northern Italian population which was equal to 0.063%. Specifically, CD was present in 12.5% of the autoimmune polyglandular syndrome (APS) type 1 cases, in four out of 60 (6.7%) of the APS type 2 cases and in one out of 40 (2.5%) of the isolated AAD cases. IgA deficiency was present in two out of 109 patients (1.8%), all of whom had normal IgG anti-gliadin. Autoantibodies to the adrenal cortex were detected in 81 out of 109 patients (74.3%).

Conclusions: In patients with AAD there is a high prevalence of both CD and IgA deficiency. Consequently, it is important to screen for CD with tissue transglutaminase autoantibodies of the IgA class and for IgA levels.

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Introduction

Autoimmune Addison’s disease (AAD) is a primary adrenal failure, caused by the destruction of the adrenal cortex by a lymphocytic aggression of the adrenals, and which develops in genetically susceptible individuals. Adrenal cortex autoantibodies (ACA) or 21-hydroxylase autoantibodies (21-OHAbs) are the markers of this condition, being present in more than 90% of patients at the onset of the disease (1).

Celiac disease (CD) is a chronic inflammation of the gut occurring in genetically susceptible individuals after the ingestion of gluten. CD may present itself with the classical clinical manifestations (the tip of the iceberg) but also includes asymptomatic patients (either silent or latent) or normal individuals (who are only genetically predisposed) representing a submerged portion of the iceberg. The silent form of CD is characterized by the presence of antibodies with manifest mucosal lesions and the latent form by the presence of antibodies with a normal mucosal morphology (2). The IgA endomysial autoantibody (IgA-EmA) or the autoantibodies to human tissue transglutaminase (IgA-tTGAbs) are the best markers with which to identify these individuals, but the diagnosis must be confirmed by means of an intestinal biopsy (3). Population-based screening studies in Europe have shown that the prevalence of CD ranges from 0.078 per 1000 births in Greece to 3.51 per
In Italy, the prevalence of CD ranges from 0.63 in the North to 1.06 in the South (4). CD is frequently associated with other autoimmune diseases and, conversely, other autoimmune diseases are frequently associated with CD (5).

The association between AAD and CD has been noted since 1970 (6). From a review of the relevant literature it can be seen that a clinical form of CD was present in 1.2–8% of the 1557 patients tested in Europe between 1967 and 1996 who already had AAD (1). Following the introduction of serological tests which can identify the asymptomatic patients in the population, two studies of cohorts of patients with AD were published. The first study examined 44 patients from Ireland with AAD, three of whom (6.8%) were known to be patients with CD prior to the study; two other (4.6%) patients were found to have IgA-EmA. A small bowel biopsy confirmed the diagnosis of CD in both cases. Among the patients with AAD, either clinical or latent CD was cumulatively present in 11.4% of the cases in Ireland (7). The second study evaluated 76 patients with AAD from Norway, using the IgA-EmA and IgA-tTGAbs. One patient was affected by CD before the study (1.3%), but five others (6.6%) were found to be positive for the IgA-EmA and/or IgA-tTGAbs and all were found to have CD following biopsy. Patients with AAD are affected by either clinical or latent CD in 7.9% of the cases in Norway (8).

The European Society for Immunodeficiencies defines IgA deficiency as a value of less than 0.07 g/l (9) (www.esid.org). IgA deficiency is more frequent in patients with autoimmune diseases: it has been described in 2–3% of those with CD (5) and in about 3% of patients with type 1 diabetes mellitus (10).

An IgA deficiency was described in one patient with AAD, in association with premature ovarian failure and chronic thyroiditis (11) and in two patients with AAD associated with CD (12). However, the real prevalence of this disorder in a cohort of patients with AAD has not yet been established.

The aim of the present work was to study the prevalence of CD (either clinical, silent or latent) in a cohort of Italian patients with AAD using the IgA-tTGAbs test, together with the prevalence of IgA deficiency.

**Subjects and methods**

**Patients**

We studied 109 patients affected by AAD, all of whom come from North Eastern Italy. Seventy-four were females and thirty-five were males (F/M = 2.1). Eight were affected by autoimmune polyglandular syndrome (APS) type 1, 61 by APS type 2 (12 of these also had type 1 diabetes mellitus) and 40 of them had isolated AAD. The mean duration of the AAD was 7.8 years (range 0–46). The mean age was 42.7 years (range 11–87 years). One hundred and five were adults and four were children. The criteria of inclusion in the group of autoimmune patients were the identification of ACA/21-OHAb and/or normal or atrophic adrenals by means of computerized tomography or magnetic resonance, plus the presence of other autoimmune diseases and a negative personal history concerning either tuberculosis or cancer. Informed consent was obtained from all the patients (from their parents in the case of the children), and the investigation was approved by the Ethics Committee of our Hospital.

**Autoantibody studies**

IgA-tTGAbs were measured in the sera of each patient using a quantitative ELISA (QUANTA Lite; Inova Diagnostics Inc., San Diego, CA, USA) (normal values < 20 U). The sensitivity and specificity of this method are more than 95% as previously described (13).

All the sera were also tested for adrenal cortex autoantibodies by means of indirect immunofluorescence, using normal human adrenal glands and for 21-OHAb by means of an immunoprecipitation assay, as previously reported (14).

**IgA determination**

All the sera were tested for levels of IgA using nephelometry (DADE Behring; Marburg, GmbH, Germany) (normal values 0.7–4 g/l). In the case of the IgA deficiency, in accordance with the European Society for Immunodeficiencies (values <0.07 g/l), the IgG anti-gliadin was measured by means of an ELISA (Gliadin IgG; QUANTA Lite; Inova Diagnostics Inc., San Diego, CA, USA) (normal values < 20 U).

**Endoscopy**

Patients with IgA-tTGabe were invited to undergo upper gastrointestinal endoscopy. Three to five fragments of distal duodenum mucosa were taken for conventional histological examination and were submerged in buffered formalin. The histological evaluation included the villous height, the crypt length and the intraepithelial lymphocyte count. The results are reported according to Marsh (15) and the guide lines for the diagnosis of CD (16).

**Genetic studies**

The patients were HLA typed for HLA DRB1*, DQA1* and DQB1* by means of molecular biology with the PCR-sequence specific primers method, using commercial kits (Genovision: Olerup SSP, Saltsjöbaden, Sweden; Dynal, Biotech Ltd., Bromborough, Wirral, UK). Genomic DNA was extracted from 200 μl peripheral blood, using the QIAMP DNA blood mini kit (QIAGEN, Hilden, Germany).

The haplotypes were deduced by means of the known patterns of linkage disequilibrium in the Italian
The DQ2 and DQ8 heterodimers are defined as follows: the DQ2 is encoded by DQA1*05 and the DQB1*02 and DQ8 are encoded by DQA1*03 and DQB1*0302 (17).

**Statistical analysis**

The differences between the various cohorts of subjects were evaluated using contingency tables with the Pearson chi-square test, with the Yates correction and a value of $P < 0.05$ was considered to be significant.

**Results**

Out of 109 patients with AAD, two (1.8%) were affected by an already diagnosed form of CD at the beginning of the study and were on a gluten-free diet, as confirmed by the negativity of the IgA-tTGAbs (see Table 1, cases 1–2). Specifically, case 1 is a 19-year-old male with APS type 2. He developed type 1 diabetes mellitus at 8 years of age, CD at 13 and AAD at 18. Case 2 is a 49-year-old female who developed AAD at 46 years of age and CD at 48. The HLA was performed on one of these patients and revealed the presence of DQ2 (Table 1). Out of the remaining 107 patients, four (3.7%) were found to be positive for the IgA-tTGAbs (see Table 1, cases 3–6), one out of eight patients (12.5%) with APS type 1, three out of 60 (5%) with APS type 2 and none with isolated AAD.

To be specific, CD was present in the clinical, silent or latent forms in six out of 109 (5.4%). This prevalence of the illness was significantly higher ($P = 0.0001$) than that reported for the Northern Italian population and was equal to 0.063% (4). CD was present in 12.5% of APS type 1 cases, in four out of 60 (6.7%) of APS type 2 cases and in one out of 40 (2.5%) of the cases of isolated AAD.

The values concerning the autoantibodies, the main clinical features of the illness and the therapy for these patients are summarized in Table 1. Three out of four (cases 3, 5 and 6) agreed to undergo endoscopy and biopsies of the distal duodenum were also taken. The fourth patient (case 4) refused to undergo these tests as she was 87 years of age and had no symptoms or signs of CD.

A histopathological pattern of the CD was demonstrated in one patient (case 3); this patient only had periodical epigastralgia. Two other cases (cases 5 and 6) displayed a normal mucosa.

The prevalence of clinical CD in patients with AAD was therefore 1.8%, the prevalence of silent CD was 0.9% and 2.7% of the cases were affected by latent CD. Adrenal cortex antibodies (ACA) and/or 21-OHAb were found in 81 out of 109 (74.3%) of the patients.

**Table 1** A summary of the clinical immunological and genetic data of the patients.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Sex (age)</th>
<th>t-TGAbs (n.v. &lt; 20 U)</th>
<th>CD</th>
<th>IgA levels</th>
<th>ACA or 21-OHAb</th>
<th>Other abs</th>
<th>Diseases in order of appearance</th>
<th>HLA</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M (19)</td>
<td>10</td>
<td>Known</td>
<td>2.4</td>
<td>Negative</td>
<td>Thyroid abs</td>
<td>Type 1 DM</td>
<td>HT AD</td>
<td>N.T.</td>
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</tr>
<tr>
<td>2</td>
<td>F (48)</td>
<td>20</td>
<td>Known</td>
<td>2.61</td>
<td>Positive</td>
<td>IFAb</td>
<td>AD</td>
<td></td>
<td>DRB1*03</td>
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</tr>
<tr>
<td>3</td>
<td>F (15)</td>
<td>211</td>
<td>Silent</td>
<td>2.4</td>
<td>Positive</td>
<td>Thyroid abs</td>
<td>HT AD</td>
<td></td>
<td>DRB1*04</td>
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<tr>
<td>4</td>
<td>F (87)</td>
<td>39</td>
<td>N.T.</td>
<td>2.85</td>
<td>Positive</td>
<td>Thyroid abs</td>
<td>HT AD</td>
<td>Chondrocalcinosis</td>
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<tr>
<td>5</td>
<td>F (25)</td>
<td>71</td>
<td>Latent</td>
<td>1.22</td>
<td>Positive</td>
<td>IFAb</td>
<td>ICA</td>
<td>GADAb</td>
<td>Chronic candidiasis</td>
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<td>DRB1*04</td>
</tr>
<tr>
<td>6</td>
<td>F (18)</td>
<td>23.5</td>
<td>Latent</td>
<td>4.29</td>
<td>Positive</td>
<td>Thyroid abs</td>
<td>PCA</td>
<td>ICA</td>
<td>GADAb</td>
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<td>DRB1*03</td>
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</table>

PCA, parietal cell autoantibodies; IFAb, intrinsic factor autoantibodies; ICA, islet-cell autoantibodies; GADAb, glutamic acid decarboxylase autoantibodies; DM, diabetes mellitus; HT, Hashimoto’s thyroiditis; n.v., normal value; N.T, not tested.
Seven out of eight (87.5%) of the patients with APS type 1 were found to be ACA positive; the only negative patient had been suffering from AAD since the age of 17: 46 out of 61 (75.4%) patients with APS type 2 were found to be positive for ACA, the mean duration of the disease in the AAD-negative cases was 16.1 years; 28 out of 40 (70%) of the patients with isolated AAD were found to be ACA positive and the duration of the disease in the ACA-negative cases was 9.1 years. Some of the ACA patients who were negative at the time of this study had been positive in previous tests. In two out of 109 (1.8%) patients with AAD, an IgA deficiency was documented. The prevalence was not significantly increased (P = 0.09) with respect to that detected in the general population where it is present in about 0.17%.

The HLA of one of the two patients with previously diagnosed CD and AAD was typical of CD (DQ2), the genetic HLA was not performed in the other patient. The HLA in the patient with the AAD and the silent form of CD was also typical of CD (DQ2-DQ8). The patients with the latent form of the disease presented with at least one of the two heterodimers predisposed to CD (see Table 1). The heterodimers were cis-encoded in all of the patients.

Discussion

AAD is the organ-specific autoimmune disease which is most prone to the development of an APS. In fact, in about 50% of these patients, other autoimmune diseases developed during their lives (1). The association between AAD and CD has been established since 1970 (6). A review of the literature on 1557 European patients with AAD, collected from 1967 to 1996, shows that a clinical form of CD was present in 1.2–8% of the cases (1). Two recent studies of cohorts of patients from Northern Europe with AAD have demonstrated that the prevalence of CD (either clinical, subclinical or latent) ranges from 7.9 to 11.4% of the population (7, 8).

Our study, the largest performed so far on a group of patients with AAD, has demonstrated that in Italian patients CD is present either in the clinical or silent form in 2.7% and in another 2.7% as the latent form of the disease, reaching an overall prevalence of 5.4%. Furthermore, we demonstrated that a gradient of positivity exists in patients with AAD that is higher in those with APS type 1, medium in those with APS type 2 and low in those with isolated AAD.

These data revealed that the association between AAD and CD varies in different countries and, that in the Southern European population, the prevalence of CD is less frequent than that observed in the Northern European population (7, 8). Nevertheless, the prevalence of CD in AAD in Northern Italy is highly significant: it is about 50-fold higher than the prevalence of CD in the general Northern Italian population, and is estimated to be 0.63 per 1000 births (4).

From our study it emerged that high titers of IgA-tTGAbs are correlated with the disease; on the contrary, low titers can identify patients with a normal duodenal mucosa as has been confirmed by others (18). Furthermore, it could be important to follow-up, at yearly intervals, the individuals who are positive for IgA-tTGAbs especially if they are genetically predisposed to CD. On the contrary, in the negative patients with AAD the test should be repeated every 2–4 years.

In general, patients with AAD take physiological doses of corticosteroid therapy in order to correct their adrenocortical insufficiency. It might be interesting to know whether, in a patient with AAD plus IgA-tTGAbs with a normal mucosal infiltration, the intake of an overdose of steroid replacement therapy could reduce the mucosal lymphocytic infiltration, thus determining a false negative evaluation of the duodenal biopsy.

It has been suggested that the precocious diagnosis of CD and the introduction of a gluten-free diet could be important features in the prevention of thyroid or pancreatic autoimmune diseases (6, 19, 20). Resulting from an analysis of the literature (7, 8) and including our data, seven cases were described as having previously identified CD and AAD at the beginning of the study. Of these seven, six had developed CD before AAD and were under a gluten-free diet. This seems to indicate that the gluten-free diet does not modify the natural history of AAD.

In the cases with AAD, the HLA pattern was characterized by haplotype DR3 (DQB1*03, DQA1*0501 and DQB1*02) and/or DR4 (DRB1*04, DQA1*03 and DQB1*0302). In the cases with CD the HLA pattern was characterized by DQ2 (DQA1*05 and DQB1*02) and in DQ2-negative patients, the pattern was DQA1*03 and DQB1*0302 (DQ8). In our patients, the two heterodimers predisposing to CD, DQ2 and DQ8, were cis-encoded and were in linkage disequilibrium with DRB1*03 and DRB1*04 respectively. The haplotypes associated with AAD therefore include the haplotypes predisposing to CD, which explains why AAD patients present with CD more frequently.

Our study estimated, for the first time, the real prevalence of IgA deficiency in a population with AAD (1.8%). In reality, this prevalence was 11-fold higher than that presumed to exist in the general population and was estimated to be 0.16% (9). This datum confirms the link between IgA deficiency and autoimmune diseases.

In conclusion, in patients with AAD, there is a high prevalence of patients with both CD and IgA deficiency. This confirms the importance of screening for these diseases when considering this cohort of patients.
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References

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