Screening for celiac disease in Tunisian patients with Graves’ disease using anti-endomysium and anti-tissue transglutaminase antibodies

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SUMMARY
Objective — Celiac disease (CD) can be associated with autoimmune thyroid diseases. The aim of this study was to screen for CD in patients with Graves’ disease in Tunisia. Patients and methods — Sera from 161 patients with Graves’ disease were tested for IgA class anti-endomysium antibodies (AEA) using indirect immunofluorescence on cryostat sections of human umbilical cord and for IgA class anti-human tissue transglutaminase antibodies (AtTG) by ELISA. Results — AEA were positive in 6 out of 161 (3.7%) patients with Graves’ disease and all 6 patients were also positive for AtTG. Four of these 6 patients with positive serological markers of CD underwent duodenal biopsy; three had marked villous atrophy, one has normal histological picture and two did not agree to undergo biopsy. The prevalence of biopsy confirmed CD in patients with Graves’ disease was 1.86% (3/161). Conclusion — Patients with Graves’ disease are at substantial risk of CD and therefore antibody screening for CD may be included in the work-up of these patients. Either AEA or AtTG may be used.

RéSUMÉ
Dépistage de la maladie coeliaque chez des patients tunisiens atteints de la maladie de Basedow en utilisant les anticorps anti-endomysium et anti-transglutaminase tissulaire
Amani MANKAI, Molka CHADLI-CHAIEB, Fathia SAAD, Leila GHEDIRA-BESBES, Mohamed OUERTANI, Habib SFAR, Monia LIMEM, Majda BEN ABDESSALEM, Moncef JEDDI, Larbi CHAIEB, Ibtsissem GHEDIRA
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Objectif — La maladie coeliaque (MC) peut être associée aux thyroidites autoimmunes. L’objectif de cette étude est de déterminer la prévalence de la MC chez des malades tunisiens atteints de la maladie de Basedow. Matériel et méthode — Notre étude a porté sur 161 sérum de malades atteints de la maladie de Basedow. La recherche des anticorps anti-endomysium d’isotype IgA (AAE) a été effectuée par la technique d’immunofluorescence indirecte sur coupe de cordon ombilical humain. Les anticorps anti-transglutaminase tissulaire d’isotype IgA (AtTG) ont été recherchés par la technique ELISA. Résultat — Six malades parmi 161 avaient des AAE et AtTG positifs. Quatre de ces 6 malades ont eu une biopsie duodénale qui a montré une atrophie villositaire dans 3 cas et une muqueuse normale dans un cas. Les deux autres malades ont refusé la biopsie. La prévalence de la MC confirmée par biopsie chez ces malades atteints de la maladie de Basedow était de 1,86 % (3/161). Conclusion — La prévalence de la MC est anormalement élevée dans la maladie de Basedow et justifie un dépistage dans cette population de malades.

Introduction
Many autoimmune diseases can be associated with celiac disease (CD) including autoimmune thyroid diseases (AITD) [1-3]. The association between CD and autoimmunity in general has been attributed to the fact that celiac patients share some HLA antigens with subjects affected by other autoimmune diseases [e.g. B8, DR3, DQW2]. The occurrence of both clinical and subclinical AITD is increased in CD [4, 5]. Hakanen et al. [4] found a frequency of 3.8% of Graves’ disease in patients with CD. Moreover, an increased prevalence of CD (3%) has been found in patients with AITD [6-15]. The true prevalence of CD in Graves’ disease is not well known. In fact, most studies included a small number of patients with Graves’ disease [8,11,14]. For these authors, patients with Hashimoto’s thyroiditis seem to have a higher risk of developing CD than patients with Graves’ disease. The most interesting study is that of Ch’ng et al. [15] who have included only patients with Graves’ disease.

The prevalence of CD in patients with Graves’ disease is not known in Tunisia. The aim of this study was to define the prevalence of CD in a large series of patients with Graves’ disease. For this purpose, we screened patients with IgA class anti-endomysium antibodies (AEA) and IgA class anti-tissue transglutaminase antibodies (AtTG).

Patients and methods

Studied population

Between January 2004 and July 2005, we studied 161 consecutive patients (43 males, 118 females, median age 32 years, range 10-83 years) with Graves’ disease. The diagnostic criteria for Graves’ disease were...
clinical signs of hyperthyroidism, elevated free thyroid hormones, suppressed thyrotrphin levels, significant titers of TSH receptor antibodies and/or thyroid peroxidase antibodies, with or without signs of thyroid associated ophthalmopathy.

Serological markers of CD

Sera from patients with Graves’ disease were tested for AEA and AtTG.

DETERMINATION OF ANTIENDOMYSIUM ANTIBODIES

We used the technique described elsewhere [16, 17]. AEA titers were measured by indirect immunofluorescence using unfixed cryosections (4 µm thick) of human umbilical cord. The starting serum dilution was chosen as 1/10. The cryosections were incubated with the diluted serum samples for 30 min. Thereafter the slides were gently washed and rinsed in phosphate-buffered saline (pH = 7.2) before incubation with fluorescein-labeled anti-human IgA antibodies (Bio-Rad ®, Marnes La Coquette, France). A positive result of AEA was recorded if a connective tissue surrounding the muscle cells fluoresced brightly in a honeycomb pattern.

DETERMINATION OF ANTI-TISSUE TRANSGlutaminase ANTIBODIES

AtTG were detected by ELISA using human recombinant tissue transglutaminase (Orgentec Diagnostika, Mainz, Germany)

Intestinal biopsy

Patients’ positive for AEA and AtTG were informed of their high probability of having CD and were offered gastrointestinal endoscopies with duodenal biopsies. Two or three mucosal biopsies were obtained. The specimens were read by two pathologists. The study was approved by Local Ethics Committee and all patients gave their informed consent.

Statistical analysis

SPSS program was used to determine median age and sex ratio.

Results

AEA were positive in 6 out of 161 (3.7%) patients with Graves’ disease. All 6 of patients had either AEA or AtTG alone. The frequency of AEA and AtTG was higher in males (6.9%, 3/43) than in females (2.5%, 3/118) but the difference was not statistically significant (p = 0.19). The median age of these six patients was 24 years (range: 16-33).

Four of these six patients with positive serological markers of CD underwent duodenal biopsy. Three patients had marked villous atrophy with increased intra-epithelial lymphocytes, one had a normal histologic picture without increased intra-epithelial lymphocytes and two did not agree to undergo biopsy. The prevalence of biopsy-confirmed CD in patients with Graves’ disease was 1.86% (3/161). The direct enquiry has revealed a history of clinical signs suggestive of CD in our 6 patients. Diarrhoea was present in 3 patients, iron deficiency anaemia in 3 and weight loss in 4. In this study, for 4 out of 6 patients with CD, screening of CD has been performed at the time of diagnosis of Graves’ disease, and for the two others, Graves’ disease has been diagnosed before antibody screening for CD. A gluten-free diet was prescribed for our three patients with biopsy-proven CD since three months.

The characteristics of these six patients with AEA and AtTG are described in table I and table II.

Discussion

The prevalence of AEA in our patients with Graves’ disease (3.7%) was significantly higher than that found in our previous study on healthy blood donors (0.28%) [18]. While the prevalence of serological markers for CD in our study was 3.7%, we could not determine the prevalence of CD. Thus, among 161 patients with Graves’ disease included in the study, 3 had biopsy-proven CD, while in two patients with AEA and AtTG, biopsy could not be performed; the patient with normal histological finding at intestinal biopsy has not yet undergone a control biopsy. Several studies have shown an almost 100% positive predictive value of AEA [19, 20]. Moreover, the specificity of AEA tested on human umbilical cord was also 100% in previous studies [16, 17, 21, 22]. Furthermore, the specificity of the human tissue transglutaminase-based ELISA is high [23, 24]. For our patient n°1, only two biopsies were obtained at endoscopy and it has been suggested that small bowel biopsy could be occasionally negative because of patchy mucosal changes [25]. On the basis of these data, our two patients who did not have a biopsy and the one awaiting control biopsy probably have, CD. According to these data, it is highly likely that the prevalence of CD is 3.7% in our patients with Graves’ disease, which is concordant with the 4.5% prevalence reported by Ch'ng et al. [15].

We did not measure total IgA levels in sera of our patients with Graves’ disease. Although CD occurs with increased frequency in those with selective IgA deficiency, screening studies of the general population suggest that very few cases will be missed by not routinely measuring IgA levels as part of the screening regimen. Thus, the strategy of routinely determining serum IgA levels or adding IgG based serology as part of a panel to screen asymptomatic individuals in the general population is not warranted [26].

In our study, of the 6 patients with Graves’ disease found to have CD, 3 were females. Considering the M/F ratio of our patients, we had a higher prevalence in males than in females (6.9%, versus 2.5%). However, this difference was not statistically significant. Volta et al. [10] have screened CD in 220 patients with Hashimoto’s thyroiditis. Seven patients were found to have CD (3.2%), and the prevalence was higher in males (8.3%) than in females (2.6%). Moreover, in the study of Larrizza et al. [11], the frequency of CD was higher in males withAITD than in females (2 of 12 vs. 5 of 78).

From a clinical point of view, the direct enquiry has revealed a history of clinical signs suggestive of CD in our 6 patients. Diarrhoea was present in 3 patients, iron deficiency anaemia in 3 and weight loss in 4. Furthermore, 5 of 6 patients have family members with autoimmune diseases. Viljamaa et al. [27] have, since 1983, screened for CD in risk groups such as patients with autoimmune diseases and first-degree relatives of celiac patients. In our study, patient n°4 has not been screened in childhood when he had type 1 diabetes mellitus (T1DM), nor when he had diarrhoea and weight loss. Although CD is present in a sister and a brother of patient n°5 who described diarrhoea since she was a child, CD has not been screened in childhood and the diagnosis was delayed until she had Graves’ disease during our screening study. Hummel et al. [28] have demonstrated that CD is frequent in first-degree relatives of patients with T1DM. The mother of patient n°1 and the brother of patient n°3 have T1DM.

ABBREVIATIONS:

CD : celiac disease
AEA : anti-endomysium antibodies
AtTG : anti-tissue transglutaminase antibodies
AITD : autoimmune thyroid diseases
T1DM : type 1 diabetes mellitus
CD had not been screened in these patients with 1st-degree relatives with T1DM despite the presence of weight loss and anaemia; in addition serological screening for CD has not yet been performed in family members with T1DM.

Unfortunately, the follow-up of our three patients with biopsy-proven CD after institution of gluten-free diet is so far too short to assess the impact of gluten withdrawal on autoimmune thyroid dysfunction or on signs of CD. In the study of Valentino et al. [8], 5 of 150 patients with AITD were found to have CD. All patients were prescribed a gluten free diet, but only 3 with Hashimoto’s thyroiditis accepted. The latter subjects showed an excellent serological, histological and clinical response at 6 months, their serum AEA disappeared; an improvement of symptoms referred to hypothyroidism as well as a reduction of the L-thyroxine dosage were noted. Viljamaa et al. [27] have studied the benefits of serological screening for CD in asymptomatic individuals. They demonstrated that long term dietary compliance in screen-detected patients was good, quality of life and bone mineral density were comparable with those in non celiac subjects and the general population. For these authors, active screening in CD risk groups seems to be reasonable rather than harmful.

In this study, for 4 of 6 patients with CD, screening for CD was performed at the time of diagnosis of Graves’ disease, and for the two others, Graves’ disease has been diagnosed before antibody screening for CD; but we can not rule out the possibility that CD coexisted from the outset of Graves’ disease or preceded

### Table I.

<table>
<thead>
<tr>
<th>N°</th>
<th>fT4</th>
<th>TSH</th>
<th>Anti-TSH-receptor</th>
<th>TPO-Ab</th>
<th>TG-Ab</th>
<th>AEA</th>
<th>AtTG</th>
<th>Intestinal biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt; 63</td>
<td>&lt; 0.15</td>
<td>6</td>
<td>—</td>
<td>&gt; 1000</td>
<td>+++</td>
<td>100</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>17.6</td>
<td>&lt; 0.15</td>
<td>9</td>
<td>&gt; 500</td>
<td>&gt; 1000</td>
<td>+++</td>
<td>100</td>
<td>STVA</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>&lt; 0.15</td>
<td>&gt; 40</td>
<td>350</td>
<td>130</td>
<td>+++</td>
<td>100</td>
<td>TVA</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 90</td>
<td>&lt; 0.15</td>
<td>22</td>
<td>190</td>
<td>180</td>
<td>+++</td>
<td>200</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 54</td>
<td>&lt; 0.05</td>
<td>&gt; 40</td>
<td>415</td>
<td>—</td>
<td>+++</td>
<td>100</td>
<td>STVA</td>
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<tr>
<td>6</td>
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<td>&lt; 0.05</td>
<td>—</td>
<td>300</td>
<td>—</td>
<td>+++ &gt; 200</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

ND: not determined. STVA: subtotal villous atrophy. TVA: total villous atrophy.

Normal ranges: $fT4$ : 7-19 pg/mL, TSH: 0.25-4.54 mUI/L, anti-TSH-receptor $d_2$ UI/L, TPO-Ab < 50 UI/mL, TG-Ab < 100 UI/mL, AtTG < 10 UI/mL.

### Table II.

<table>
<thead>
<tr>
<th>N°</th>
<th>Sex</th>
<th>Age at CD screening (years)</th>
<th>Age at diagnosis of Graves’ disease (years)</th>
<th>Duration of Graves’ disease (years)</th>
<th>Clinical features suggestive of CD</th>
<th>Familial history of autoimmune disease</th>
<th>Personal history of other autoimmune disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>21</td>
<td>17</td>
<td>4</td>
<td>Anemia, Weight loss</td>
<td>Mother with T1DM</td>
<td>—</td>
</tr>
<tr>
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<td>F</td>
<td>16</td>
<td>16</td>
<td>0</td>
<td>Anemia, Hypocholesterolemia</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>33</td>
<td>33</td>
<td>0</td>
<td>Anemia, Weight loss, Hypoprotidemia, Hypocalcemia, Hypoalbuminemia,</td>
<td>A brother with T1DM</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>27</td>
<td>27</td>
<td>0</td>
<td>Diarrhoea, Weight loss, Hypoprotidemia, Hypocholesterolemia,</td>
<td>Cousins with AITD</td>
<td>T1DM</td>
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<tr>
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<td>20</td>
<td>0</td>
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<td>A sister and a brother with CD</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>30</td>
<td>25</td>
<td>5</td>
<td>Diarrhoea, Weight loss</td>
<td>Graves’ disease</td>
<td>—</td>
</tr>
</tbody>
</table>

CD: celiac disease, T1DM: type 1 diabetes mellitus, AITD: autoimmune thyroid diseases.
it, especially given the fact that the 6 patients had signs suggestive of CD since they were children. One patient out of 6 has also T1DM. Not et al. [29] have demonstrated that the prevalence of autoimmune disorders in patients with both T1DM and CD is significantly higher than in subjects with T1DM alone. We did not find other associated autoimmune disorders in the other five patients. It must be however underlined that the relatively young age of these patients (33 years or less) ensures that such complications may subsequently develop. Larizza et al. [11] found a higher frequency of autoimmune disorders in patients with both AITD and CD than in those with only AITD.

Our 6 patients, who have clinical signs suggestive of CD many years ago, should have been detected several years before the diagnosis of Graves’ disease. There are multiple reasons for the diagnostic delay; these include lack of awareness of the more subtle presentations of CD, atypical presentation (absence of diarrhoea) that are in fact more common, alternative diagnosis and attribution of symptoms to other diagnosis. The results of this investigation indicate that health care professionals in Tunisia need to appreciate the necessity for screening for CD in high risk groups with the use of serological screening tests [16, 17] allowing identification of patients who could benefit from a diagnostic small-intestinal biopsy. The lack of knowledge in the primary care setting was responsible for the delay in the diagnosis of CD in our 6 patients who all have clinical signs suggestive of CD and who also have either personal or family history of another autoimmune disease including CD and T1DM. Patients with Graves’ disease are at substantial risk of CD and therefore antibody screening for CD may be included in the work-up of these patients. In fact, weight loss and diarrhoea must not be attributed to Graves’ disease. Either AEA or ATG may be used. Early screening may reduce morbidity [30] and improve quality of life.

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REFERENCES


