IgA anti-actin antibodies in celiac disease

Anticorps anti-actine d’isotype IgA au cours de la maladie cœliaque

A. Achour a, Y. Thabet a, W. Sakly a, A. Mankai a, N. Sakly a, A. Ayadi b, M.T. Sfar b, F. Amri c, A. Harbi d, A.S. Essoussi e, A. Krifa f, S. Ajmi g, I. Ghedira a,h

Research unit (03/UR/07-02), Faculty of pharmacy, 5000 Monastir, Tunisia
Paediatric department, Tahar Sfar Hospital, Mahdia, Tunisia
Paediatric department, Ibn El Jazzar Hospital, Kairouan, Tunisia
Paediatric department, Sahloul Hospital, Sousse, Tunisia
Paediatric department, Farhat Hached Hospital, Sousse, Tunisia
Internal Medicine department, Farhat Hached Hospital, Sousse, Tunisia
Gastroenterology department, Sahloul Hospital, Sousse, Tunisia
Laboratory of Immunology, Farhat Hached Hospital, Sousse, Tunisia

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Summary

Aims. — The purpose of this study was to determine the sensitivity and specificity of IgA anti-actin antibodies (IgA-AAA) for celiac disease (CD), to investigate their usefulness as a marker of compliance in CD patients to the gluten-free diet (GFD), and to assess the relationship between their presence in the sera of CD patients and severity of intestinal mucosal damage.

Patients and methods. — A total of 182 patients with CD were studied: 63 patients were untreated; 50 patients were following a strict GFD; and 69 patients were non-compliant with a GFD. IgA-AAA was detected using a homemade enzyme-linked immunosorbent assay (ELISA).

Results. — IgA-AAA showed a sensitivity of 41.3% and a specificity of 71.4% for a diagnosis of CD. In children, the frequency of IgA-AAA detection was lower in those following a strict GFD (23.1%) compared with untreated patients (39.4%) and those not complying with a GFD (32.5%). In patients following a strict GFD, IgA-AAA detection was significantly less frequent in children than in adults (23.1% vs. 58.3%, respectively; P < 0.001). IgA-AAA was found in 17 out of 52 CD patients with total villous atrophy (32.7%), and in one out of 11 patients with subtotal villous atrophy (9%).

Conclusion. — IgA-AAA cannot replace anti-endomysium and anti-tissue transglutaminase antibodies in the diagnosis algorithm of CD, but it can serve as a reliable marker of severe intestinal mucosal damage in CD patients.

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Introduction

Celiac disease (CD) is an immune-mediated enteropathy that is triggered, in genetically susceptible individuals, by the ingestion of gluten, which is found in wheat, and in similar proteins present in barley and rye [1]. It is characterized by inflammation of the small bowel mucosa, with varying degrees of intestinal villous atrophy, crypt hyperplasia and an increased number of intraepithelial lymphocytes [2]. Therapy involves a gluten-free diet (GFD) in which these offending grains are eliminated [3]. Nowadays, it is evident that CD is one of the most common underdiagnosed diseases in general practice, with a frequency of around 1% in the general population [4–6]. CD is associated with a wide variety of other autoimmune diseases, such as type 1 diabetes mellitus [7,8], Graves' disease [9] and Addison's disease [10]. Malignancy has also been observed in CD patients with a higher frequency than in the general population [11], and osteomalacia has been described as a major feature of CD in adults [12].

The use of serological markers has revolutionized the screening and diagnosis of CD [13]. IgA anti-endomysium antibodies (IgA-EmA) and IgA anti-human recombinant tissue transglutaminase antibodies (IgA-AtTG) have high sensitivity and high specificity for CD [14,15]. Recently, there have been reports of encouraging results for antibodies that bind to synthetic deamidated gliadin peptides. Both isotypes (IgA and IgG) of these antibodies have been shown to be highly sensitive and specific for active CD [16,17].

Clemente et al. [18] described a high frequency of IgA antibodies against actin filaments (IgA-AAA) in celiac patients. Actin is known to be a major component of the cytoskeleton of almost all eukaryotic cells. It is a globular protein found in either monomeric form as G-actin or in filamentous form as F-actin, and each actin subunit binds to either ATP or ADP [19,20].

As only a few studies [18,21,22] have reported on the frequency of IgA-AAA as determined by enzyme-linked immunosorbent assay (ELISA) in CD, and no data are available for Tunisian patients in particular, the present study aimed to assess the sensitivity and specificity of IgA-AAA for CD to determine whether or not these antibodies could be used as a marker to evaluate patients’ compliance with a GFD. Another objective was to investigate the relationship between the presence of serum IgA-AAA and severity of intestinal mucosal damage.

Patients and methods

Study population

The sera of CD patients were obtained from four hospitals in the center of Tunisia. The present study used only sera available to our laboratory that had been collected between June 1999 and March 2009. Only patients with total or subtotal villous atrophy, according to the March 2009 classification [2], were recruited into the study. In total, 182 CD patients were studied (99 children and 83 adults). Patients were divided into three groups:

- group I included 63 untreated patients (42 females [F], 21 males [M]; median age: 16 years, range: 11 months to 54 years);
- group II comprised 50 patients following a strict GFD (30 F, 20 M; median age: 24 years, range: 4 months to 34 years);
- group III was made up of 69 patients who were not compliant with a GFD (48 F, 21 M; median age: 14 years, range: 18 months to 47 years).

Table 1 shows the demographic characteristics of all of our studied CD patients.

Also, as a control population, 19 patients with primary biliary cirrhosis (PBC) and 37 with systemic lupus erythematosus (SLE) were included in the study. In addition, the sera of 52 healthy blood donors with no history of CD served to determine the cut-off point of the ELISA. All sera were stored at −80 °C until needed.

Résumé

Objectif. — Déterminer la sensibilité et la spécificité des anticorps anti-actine d’isotype IgA (AAA-IgA) au cours de la maladie cœliaque (MC), évaluer la relation entre leur présence dans le sérum de patients cœliaques et la gravité des dommages au niveau de la muqueuse intestinale, et déterminer leur utilité dans le contrôle de la compliance au régime sans gluten (RSG).

Patients et méthodes. — L’étude a porté sur 182 sérums prélevés chez des patients cœliaques. Soixante-trois patients sont non-traités, 50 sont sous RSG strict et 69 sont sous RSG mal suivi. Les AAA-IgA sont recherchés par une technique Elisa maison.

Résultats. — La sensibilité des AAA-IgA est de 41,3 % et sa spécificité est de 71,4 %. Chez les enfants, mais pas chez les adultes, les AAA-IgA sont moins fréquents chez les patients sous RSG strict (23,1 %) que chez les patients non traités (39,4 %) et ceux qui ne suivent pas leur régime (32,5 %). Chez les patients sous RSG strict, les AAA-IgA sont significativement moins fréquents chez les enfants que chez les adultes (23,1 % versus 58,3 %, p <0,001). Les AAA-IgA sont détectés dans 17 cas parmi les 52 patients cœliaques présentant une atrophie villositaire totale (32,7 %) et dans un cas sur 11 chez les patients présentant une atrophie villositaire subtotale (9 %).

Conclusion. — Les AAA-IgA ne peuvent pas remplacer les anticorps anti-endomysium et les anticorps anti-transglutaminase tissulaire pour le diagnostic de la MC. Cependant, ils constituent un marqueur fiable pour évaluer les dommages graves de la muqueuse intestinale chez les patients cœliaques.
Detection of IgA anti-actin antibodies

ELISA was performed using, as antigen, lyophilized actin from rabbit muscle (Sigma Chemicals, St. Louis, MO, USA). Briefly, the wells of the microplates (Nunc, Roskilde, Denmark) were coated with either actin (10 μg/mL) diluted with bicarbonate buffer, pH 9.6, or with bicarbonate buffer, pH 9.6, alone. The microplates were then incubated overnight at 4°C. After three washings with phosphate buffered saline (PBS), a blocking solution (PBS/bovine serum albumin [BSA] 2%) was added to each well and left for 1 h at 37°C. Serum samples were diluted at 1/100 in PBS/BSA 1%, added to the wells and incubated for 90 min at 37°C. After three washing steps with PBS, horseradish peroxidase-conjugated anti-human IgA antibody (Bio-Rad, Marnes-La-Coquette, France) diluted at 1/2500 in PBS/BSA 1% was added to each well and incubated for 60 min at room temperature. The immune reaction was revealed by adding the substrate solution. The absorbance was read at 492 nm (absorbance of sera = absorbance of wells coated with actin − absorbance of wells coated with bicarbonate buffer). The sera were only considered positive if values were greater than 0.246, which corresponds to the mean absorbance plus two standard deviations obtained using sera samples from the 52 healthy blood donors.

Statistical analysis

The χ² test was used to compare frequencies of all categorical variables. P values less than 0.05 were considered significant, and all calculated P values were two-tailed.

Results

IgA-AAA had a sensitivity of 41.3% for diagnosing CD and a specificity of 71.4% (Table 2). The frequency of IgA-AAA in patients in the disease-control group was 28.6%, with no statistically significant difference between this group and the untreated CD patients.

Frequencies of IgA-AAA were similar in the different groups of CD patients: 41.3% in untreated patients; 40% in patients following a strict GFD; and 39.1% in patients not compliant with a GFD (Table 3). In children, the frequency of IgA-AAA was lower in patients following a strict GFD (23.1%) compared with untreated patients (39.4%) and those not compliant with a GFD (32.5%). In adults, the frequency of IgA-AAA was 43.3% in untreated patients, 58.3% in those with successful adherence to a GFD and 48.3% in patients not compliant with a GFD. Also, the frequency of IgA-AAA was not statistically different between untreated children (39.4%) and untreated adults (43.3%). In patients following a strict GFD, IgA-AAA was significantly less frequently detected in children than in adults (23.1% vs. 58.3%, respectively; P < 0.001). In patients not compliant with a GFD, the frequency of IgA-AAA was not statistically different between adults (48.3%) and children (32.5%) (Table 3).

As for the presence of IgA-AAA in relation to mucosal histology in patients with untreated CD (63 patients), the antibodies were found in 17/52 patients with total villous atrophy (32.7%; 23 children, 29 adults), and in 1/11 patients (9%; eight children, three adults) with subtotal villous atrophy (Table 4).

Discussion

The optimal serological approach for the diagnosis of CD is based on EmA and AtTG assays, which are known to be highly accurate [13–15]. However, these serological tests do not correlate with histopathological features [23]. Recently, however, Clemente et al. [18] described for the first time the AAA assay in CD, and proposed it as a possible marker of severity of intestinal mucosal damage in CD.

In the present retrospective study, the frequency of IgA-AAA in a cohort of 182 CD patients was analyzed, and showed that the frequency of IgA-AAA was not statistically different between untreated CD patients (41.3%) and patients in the disease-control group (28.6%). This specificity of 71.4% was similar to that found by Carroccio et al. (72%) [22]. However, Granito et al. [21] reported a specificity of 100% compared with controls. This variation may be due to the fact that the patients in the control groups differed across these studies.

The sensitivity of IgA-AAA for CD in the present study was 41.3%. This value was similar to that found by Clemente et al. (30.5%) [18] and higher than that of Granito et al. (14.7%) [21], but lower than those reported by Carroccio et al. [22,24] (80% and 86% in two studies, respectively). This discrepancy might be explained by differences in the antigenic substrate used for the ELISA. In fact, both the
Table 2  Frequency of IgA-AAA detection in celiac patients and the control groups.

<table>
<thead>
<tr>
<th></th>
<th>Untreated CD patients</th>
<th>SLE</th>
<th>PBC</th>
<th>SLE + PBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of IgA-AAA (n/n)</td>
<td>41.3% (26/63)</td>
<td>27% (10/37)</td>
<td>31.6% (6/19)</td>
<td>28.6% (16/56)</td>
</tr>
</tbody>
</table>

CD: celiac disease; SLE: systemic lupus erythematosus; PBC: primary biliary cirrhosis.

Table 3  Frequency of IgA-AAA detection in the various celiac disease (CD) groups.

<table>
<thead>
<tr>
<th></th>
<th>Untreated patients</th>
<th>Patients following a strict GFD</th>
<th>Patients not compliant with a GFD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All CD patients</td>
<td>Children</td>
<td>Adults</td>
</tr>
<tr>
<td>Rate of IgA-AAA (n/n)</td>
<td>41.3% (26/63)</td>
<td>39.4% (13/33)</td>
<td>43.3% (13/30)</td>
</tr>
</tbody>
</table>

GFD: gluten-free diet.

a P = 0.011 (children vs adults).

The present study and that of Granito et al. [21] used monomeric actin (G-actin) as antigen, whereas Carroccio et al. [22,24] used polymerized actin (F-actin). The latter type of actin offers a greater number of potential epitopes that could be recognized by IgA-AAA than does monomeric (G)-actin [25]. This might explain the higher sensitivity of ELISA in the studies by Carroccio et al. [22,24]. Moreover, it has been demonstrated that the indirect immunofluorescence (IIF) technique is more sensitive than ELISA for the detection of IgA-AAA [21,24]. Indeed, Clemente et al. [18] achieved a sensitivity of 60.2% and 30.5% with IIF and ELISA, respectively and, in the same way, Carroccio et al. [24] achieved a sensitivity of 93% and 80% for IIF and ELISA, respectively. The higher sensitivity of IIF in comparison to ELISA could be due to the fact that the immunodominant epitopes of actin are better exposed in HEp-2 cells than in the polystyrene plate.

IgA-AAA was as frequently detected in untreated adults (43.3%) as in untreated children (39.4%). These results are in concordance with those reported by Carroccio et al. [22] (81.3% in adults and 76% in children), but not with the findings of Clemente et al. [18], which showed a significantly lower frequency of IgA-AAA assessed by IIF in children than in adults.

As for GFD, the present study had two groups of such patients: those strictly compliant and those not compliant with the GFD. This is because, in Tunisia, it is difficult to maintain a GFD for two reasons. First, the typical diet consists mainly of bread, pasta and couscous and, second, gluten-free foods are very expensive. The frequency of IgA-AAA was significantly lower in compliant children than in compliant adults (23.1% vs. 58.3%, respectively; P = 0.001). This difference may be explained by the fact that the duration of exposure to gluten is longer in adults. It has also been reported that the rate of normalization of the functional integrity of the intestinal mucosa is slower in adults than in children [26].

Recently, it has been demonstrated that zonulin expression, induced by gluten, is increased during the early stages of CD. Zonulin is a mediator that increases intestinal permeability in patients with CD, and it has been suggested that the reported changes in the tight junctions between enterocytes in the early stages of the disease are mediated by zonulin [27,28]. Modifications in the cytoskeletal organization leading to these tight junction alterations are related to protein kinase C (PKC)-dependent polymerization of actin monomers into actin filaments. These modifications, in turn, lead to disorganization of enterocyte actin filaments [29]. The question then arises: is the production of IgA-AAA due to the fact that actin filaments are polymerized, and the new exposed epitopes thus created are being recognized by the immune system?

It has been previously confirmed that serum AAA positivity is indicative of more severe intestinal histological damage. Indeed, IgA-AAA was more frequently seen in the CD patients with total villous atrophy (32.7%) than in those with subtotal villous atrophy (9%), and other, similar findings have been reported. Clemente et al. [18] found that the frequency of AAA determined by ELISA was higher in patients with severe villous atrophy (83%) than in those with moderate villous atrophy (17%). Granito et al. [21] found that AAA, as determined by IIF, was significantly less frequently found in patients with mild villous atrophy than in patients with severe villous atrophy (6% vs. 46%, respectively; P < 0.0001).

Table 4  Frequency of IgA-AAA detection according to intestinal mucosal damage in 63 untreated celiac disease (CD) patients.

<table>
<thead>
<tr>
<th></th>
<th>CD with total villous atrophy</th>
<th>CD with subtotal villous atrophy</th>
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<tbody>
<tr>
<td>Rate of IgA-AAA (n/n)</td>
<td>32.7% (17/52)</td>
<td>9% (1/11)</td>
</tr>
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</table>
Finally, in the study of Carroccio et al. [22], the frequency of AAA was significantly greater in patients with total villous atrophy than in those with partial villous atrophy (84.4% vs. 68.3%, respectively; P < 0.01).

In conclusion, IgA-AAA prevalence was 41.3% in the present study’s celiac population, with a specificity of 71.4%. In children, but not in adults, IgA-AAA levels were diminished when strictly following a GFD. In addition, IgA-AAA is a reliable marker of severe intestinal mucosal damage in CD patients, and a simple ELISA test can be used to make the determination. Nevertheless, further studies of larger populations are needed to investigate the mechanisms and significance of the production of these autoantibodies in patients with CD and other autoimmune diseases.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

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References
