Anti Enterocyte Autoantibodies in Pediatric Celiac Disease

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Abstract

Many aspects are shared between celiac disease and autoimmune enteropathy: symptoms, diagnosis by serological bio-markers, endoscopic findings, intestinal pathology, differential diagnosis, and pharmaceutical therapy in selected cases. Antibodies to tissue transglutaminase have been described in over 30% of patients with autoimmune enteropathy, but no anti-enterocyte antibodies were detected in a small population of pediatric celiac patients.

The aim of the current study was to identify anti-enterocyte antibodies in pediatric patients with well-characterized celiac disease compared to a group of children with recurrent abdominal pain.

Materials and methods: celiac disease (N=38) was diagnosed based on positive celiac serology (anti-neo-epitope tissue transglutaminase (Aeskū)’ and/or anti endomysial antibodies) and small intestinal biopsy that was consistent with celiac disease.

The comparison group consisted of age and sex matched patients (N=41) with a history of abdominal pain, negative celiac serology, normal upper endoscopy and normal small intestinal histology.

Detection of anti-enterocyte antibody was performed using Western blot. Homogenates from normal human intestinal mucosa were electrophoresed on 7.5% SDS-PAGE and transferred to nitrocellulose membranes. Blots were treated with blinded patient sera and developed using ELISA kit.

Results: In the pediatric celiac group 3/38 (8.6%) compared to 6/35 (17.1%) in the non-celiac group were positive for anti-enterocyte antibody. No statistically significant difference in the presence of anti-enterocyte antibodies in patients with celiac disease.

Conclusions: About 8% of children with celiac disease may have antibodies to enterocytes, but the frequency is not increased when compared to children with recurrent abdominal pain.

Keywords: Celiac disease; Autoimmune enteropathy; Anti-enterocyte antibodies; Anti neo-epitope tissue transglutaminase; Autoimmunity; Children

Introduction

Celiac disease (CD) is a life-long autoimmune condition [1] mainly involving the small intestine of genetically susceptible individuals. Gluten, which is the storage protein of wheat and its alcohol soluble gliadins are the offending inducers of the disease together with structurally related molecules found in barley and rye.

Tissue transglutaminase (tTg) is the auto-antigen against which the abnormal immune response is directed to [2,3] and two main auto-antibodies, anti-endomysium and anti-tTg, are the most useful serological markers to screen the disease [4,5]. Recently, three additional autoantibodies, namely anti-deamninated gliadin peptide, anti-neo-epitope TtG, and the antibody against the neo-epitope microbial transglutaminase, were found to be reliable for CD screening [6-10].

The prevalence of CD has increased in the last decades, following the trend in other autoimmune diseases [3,6,7]. Many complications including infertility and malignancy may occur in untreated CD [7-9]. Thus, early diagnosis and subsequent adherence to a gluten-free diet is highly recommended. The epidemiology and phenotype of CD are changing. The classic picture of malnutrition, chronic diarrhea and nutritional deficiencies in a young child is being replaced by older patients with extra intestinal manifestations or a paucity of symptoms. These changes make the diagnosis of the disease more difficult [8,9].

Autoimmune enteropathy (AIE) is a rare disease characterized by intractable diarrhea, villous atrophy of the small intestine, the presence of autoantibodies against the enterocyte, and in contrast to CD, a decrease in intestinal intraepithelial lymphocytes [11-13]. Patients do not respond to dietary modification, including a gluten free diet, and treatment usually includes immunosuppressive agents [14,15], or in some patients total parenteral nutrition. AIE more commonly affects infants within the first six months of life; however, the diagnosis is increasingly being recognized in adults [16].
Many aspects are shared between CD and AIE including symptoms such as abdominal pain, diarrhea and malabsorption, endoscopic findings, intestinal pathology, and even therapy in selected individuals [16,17]. Antibodies to tTg have been described in over 30% of patients with AIE [14,16]. Other immune-mediated disorders, e.g. autoimmune thyroiditis, myasthenia gravis, as well as CD may be associated with AIE [18,19]. In fact, due to clinical and histopathological features of AIE mimicking CD, patients may be misdiagnosed and treated with a gluten-free-diet without any improvement. In refractory CD one should include AIE in the differential diagnosis [20]. Left untreated, AIE can result in severe malabsorption, intestinal failure and even enteropathy-associated T-cell lymphoma is described in some patients [21]. The association between CD and AIE is further strengthened by the observation that about 20% of patients with AIE display the same immuno-histochemical and genetic features found in patients with CD [14]. AIE and CD are frequently associated with immunodeficiency disorders [11].

Based on the similarities of the two conditions, the frequency of anti-enterocyte antibodies (AEA) were measured in a well characterized, population of children with CD and in an age and gender matched comparison group of children with a negative evaluation for CD.

Materials and Methods

CD (N=38) mean age: 7.3 ± 4.3 years, F:M 3.7 were diagnosed based on positive celiac serology (anti-neo-epitope tissue transglutaminase (AESKULISA® tTg New Generation, AESKUDIAGNOSTICS, Germany) and/or anti endomysial antibodies (AESKUSLIDES® EMA, AESKUDIAGNOSTICS, Germany) and small intestinal biopsy that was consistent with CD.

Forty one children with age 7.3 ± 5.6 years, F:M 1.2 served as comparison group. They were evaluated for recurrent abdominal pain (RAP), and had negative celiac serology, normal upper endoscopy and normal small intestinal biopsies. The two groups’ age and gender were not significantly different.

AEA test was performed using western blot. Homogenates from normal human intestinal mucosa were diluted in a sample buffer (1:10), centrifuged at 1,000 g for 3 minutes and separated on 7.5% SDS-PAGE gel. Proteins were transferred to nitrocellulose membranes. All sera were blinded to the investigators performing the analysis. Blots were treated with serum from CD and comparison groups followed by mouse anti-human immunoglobulin A and immune-developed using Vectastain ABC kit. For positive control brush border enzyme - alkaline phosphatase was used. The chi-square test was used for the comparison of the two groups.

Results

<table>
<thead>
<tr>
<th></th>
<th>AEA positive</th>
<th>AEA negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>3 (3.79)</td>
<td>35 (44.3)</td>
<td>38</td>
</tr>
<tr>
<td>RAP</td>
<td>6 (7.59)</td>
<td>35 (44.3)</td>
<td>41</td>
</tr>
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( ): %; CD: Celiac Disease; RAP: Recurrent Abdominal Pain

Table 1: AEA frequency in pediatric CD compared to the abdominal pain group.

In the CD group, 3/35 (8.6%) were positive for AEA and in the non-celiac group, 6/35 (17.1%) were AEA positive (Table 1). Although the frequency of AEA positivity in the abdominal pain group was higher than in the celiac group, statistically significant levels were not reached (P=0.48).

Discussion

Although around 8% of patients with CD will have positive AEA, the present study did find that AEA were present in CD and in children with recurrent abdominal pain. Antibodies to tissue tTg have been described in a third of AIE patients [14,16]. Not all patients with AIE will have positive AEA, so it remains possible that patients with CD who might have AIE could be AEA negative. For patients with CD who do not respond to a gluten free diet, AEA negative AIE remains a possibility. Nevertheless, when Mirakian et al. published their seminal study describing the autoimmune aspects in protracted diarrhea of infancy, no AEA were detected in 10 CD children, which is consistent with the present results [22].

The explanation for the positive AEA in the children with RAP cannot be explained except by accepting that this is the false positive rate for this test. The diagnosis of AEA relies not only on the serologic testing but also on the clinical presentation and the histology. AEA remains a diagnosis that should be considered in the right clinical-pathologic setting in an individual with a positive AEA. However, AIE may occur in patients who have negative AEA, as is the case for negative tTg antibodies in CD [23].

In conclusion, despite multiple common clinical features between CD and AIE, no increased incidence of AEA was found in a pediatric CD cohort.

References

9. Lerner A, Jeremias P, Neidhöfer S, Matthias T (2016) Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable then


