Original Article

Antibody testing in Indian children with celiac disease

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Background: We prospectively evaluated the usefulness of IgA tissue transglutaminase antibodies (IgA tTG) in the initial diagnosis of celiac disease (CD), and compared its diagnostic potential with that of IgA anti-endomysial antibodies (IgA EMA) and anti-IgA and IgG gliadin antibodies (AGA and AGG, respectively). Methods: Sera of 23 untreated children fulfilling the revised ESPGHAN criteria for diagnosis of CD (Group I; mean age 10.8 y); 19 disease controls (Group II; mean age 8.5 y) presenting with chronic diarrhea, short stature or both; and 22 healthy children (Group III; mean age 8.8 y) were studied. These were tested in a blinded manner for AGA, AGG, IgA tTG (guinea pig as antigen) and IgA EMA. Results: In Group I, IgA EMA was positive in 19, IgA tTG in 17, AGA in 14 and AGG in 17 patients. In Group II, these tests were positive in 1, 0, 2 and 14 patients, respectively, and in Group III, in 0, 0, 0 and 1 child, respectively. Analyzing data from Group I and II, IgA EMA, IgA tTG, AGA and AGG had sensitivity rates of 83%, 74%, 61% and 74%, respectively; the specificity rates were 95%, 100%, 89% and 26%; positive predictive values were 95%, 100%, 88% and 55% and negative predictive values were 82%, 74%, 65% and 45%, respectively. Conclusion: IgA tTG is useful for the diagnosis of CD, with sensitivity and specificity rates comparable to those of EMA, and this test is well suited for use in tropical countries like India. [Indian J Gastroenterol 2006;25:132-135]

Celiac disease (CD) is increasingly being recognized in India.1,2 The diagnosis of CD is based on the presence of symptoms, demonstration of abnormal small bowel histology, and unequivocal clinical response to gluten-free diet (GFD). Positive serology, though not essential for the diagnosis according to the European Society for Paediatric Gastroenterology and Nutrition (ESPGHAN) criteria, makes the diagnosis more certain.3

In tropical regions, several other diseases with chronic diarrhea, viz., tropical sprue, giardiasis and malnutrition, may be associated with varying degrees of villous atrophy, and hence need to be distinguished from CD.4,5 There is also a need for a good screening test so as to select subjects who merit biopsy. Several serological tests have evolved over the last two decades for this purpose. Of the available tests, anti-endomysial antibodies (IgA EMA) have a good specificity and sensitivity for the diagnosis of CD.6 However, detection of EMA uses an indirect immunofluorescence assay and is thus operator-dependent, expensive, and prone to errors and delay. Recently, tissue transglutaminase (tTG) has been shown to be the major auto-antigen target for EMA.7 This led to the development of enzyme immunoassays (EIAs) for the detection of IgA antibodies against tTG; these tests are simple, cheap and operator independent. In studies from the West, tTG antibodies had sensitivity and specificity rates similar to those of EMA.8

The primary aim of this study was to determine the usefulness of IgA tTG antibodies in the initial diagnosis of CD and its differentiation from other enteropathies in India. The secondary aim was to compare the usefulness of IgA tTG with that of IgA EMA and antigliadin antibodies (IgA [AGA] and IgG [AGG]).

Methods

We used stored sera obtained from 23 children (15 boys; median age 11 [range 3-18] years) with CD [Group I] attending the Pediatric Gastroenterology services of our institution between December 1991 and December 2001. The diagnosis of CD was based on the revised ESPGHAN criteria.3

We included two control groups. The first of these (Group II) included 19 disease controls (median age 11 [range 5-15] years; 10 boys) who presented between years 1998 and 2001 with chronic diarrhea, short stature or both, and in whom a diagnosis of CD was clinically suspected but later ruled out. These children had either normal small bowel histology, or partial villous atrophy without clinical response to GFD for >6 months, or other known etiology of chronic diarrhea. The other control group (Group III) included 22 healthy children (median age 9 [3-14] years; 13 boys) who neither had symptoms suggestive of CD, nor had a risk factor for increased risk of CD, viz., diabetes mellitus, Down’s syndrome, hypothyroidism, a first-degree relative with CD, etc; these children did not undergo duodenal biopsy.
For Groups I and II, sera had been collected before duodenal biopsy. For Group III, these were collected just before the study. All sera were stored at -80°C. Children with IgA deficiency were excluded. The study was approved by our institution’s Ethics Committee; informed consent was obtained from parents of all the study children.

Serological tests
The stored sera were tested by laboratory technicians, who were unaware of the clinical details and group assignment of the patients. AGA, AGG and IgA tTG were tested using commercially available EIAs (Binding Site, Birmingham, England); the method followed for detection of IgA anti-tTG antibodies was similar to that for detection of AGA except that micro wells were pre-coated with calcium-activated guinea pig tTG antigen. Borderline EIA values for AGA, AGG and IgA tTG were taken as negative.

IgA EMA was tested using an indirect immunofluorescence assay. Frozen sections from monkey esophagus were used as the substrate and the characteristic staining of connective tissue around smooth muscle fibers was looked for. Appropriate positive and negative controls were used in each run.

Small bowel biopsy and histological analysis
Endoscopic duodenal biopsy sections (4-6 tissue pieces per patient) of study subjects were assessed by experienced pathologists, who were unaware of the antibody assay results. The biopsies were evaluated for villous atrophy, crypt hyperplasia, increased intraepithelial lymphocytes and infiltration of the lamina propria, and graded using the Marsh classification.

Results of various serological tests in three groups of children are shown in the Table. Distribution of optical density values of AGA, AGG and IgA tTG in

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Celiac disease</td>
<td>Disease controls</td>
<td>Healthy controls</td>
</tr>
<tr>
<td>Number</td>
<td>23</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Antigliadin antibody positive</td>
<td>IgA 14 (61%)</td>
<td>2 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>IgG</td>
<td>17 (74%)</td>
<td>14 (74%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>IgA tissue transglutaminase positive</td>
<td>17 (74%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgA anti-endomysial antibodies positive</td>
<td>19 (83%)</td>
<td>1 (5%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Statistical analysis
Standard formulae were used for calculating sensitivity, specificity and overall accuracy. The gold standard for calculation of sensitivity, specificity and accuracy were confirmed cases of CD (Group I) evidenced by small bowel biopsy suggestive of CD at diagnosis and a definite response to GFD.

Results
Of 23 children with CD, 21 (91%) had short stature, 20 (87%) had anemia and 19 (93%) had chronic diarrhea. Seventeen (74%) patients had subtotal villous atrophy (Marsh IIIb) and 6 (26%) had partial villous atrophy (Marsh IIIa). All cases showed rapid symptomatic response and substantial improvement in growth on GFD. Over a median follow up of 11 months, height standard deviation (Z) scores improved from median -4.40 to -3.60 and weight-for-height percentage from median 84.5 to 104.

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Fig: Antibodies in celiac disease patients, disease controls and healthy controls. Line indicates the cut-off between specimens testing positive and negative in terms of optical density value. Left: IgA antgliadin antibodies; Middle: IgG antgliadin antibodies; Right: IgA tissue transglutaminase antibodies
sera of all children are shown in the Figure.

Of the 19 cases in Group II, 15 (79%) had chronic diarrhea, 10 (58%) had short stature and 9 (42%) had anemia. Of these 19 children, 17 had normal duodenal histology and one had histological features suggestive of lymphangiectasia. The remaining one child had partial villous atrophy (Marsh IIIa); this child tested positive only for AGG but not for other antibodies, showed no response to GFD for 6 months, and later was diagnosed to have human immunodeficiency virus (HIV) infection. Tests for malabsorption (stool fat by Sudan staining and D-xylose test) were done in 16 cases; D-xylose test alone was done in one other child. Of these, stool fat and D-xylose were both abnormal in 6 cases, D-xylose alone in 5, fecal fat in 3; two other cases had normal tests. A definite final diagnosis was obtained in 11 cases (giardiasis 3, HIV infection 2, bacterial overgrowth 2, abdominal tuberculosis 1, lymphangiectasia 1, megaloblastic anemia 1, and Turner's syndrome 1). In 8 children, no definite diagnosis was reached. In this group, only one patient tested positive for IgA EMA. IgA tTG was negative in all patients. The subject with positive IgA EMA had normal biopsy and could be labeled as a potential CD subject.

In Group III, all subjects were negative for IgA EMA, IgA tTG and AGA. Only one subject was positive for AGG. The results of IgA EMA and IgA tTG were identical (i.e., both positive [n=16] or both negative [n=43]) in 92.2% (59/64) of subjects.

The sensitivity, specificity and accuracy of IgA EMA and IgA tTG were 82.6%, 94.7%, 88.1% and 73.9%, 100%, 85.7%, respectively when calculated for subjects in Groups I and II. AGA and AGG had lower sensitivity (60.8% and 73.9%), specificity (60.8% and 26.3%) and diagnostic accuracy (73.8% and 52.4%), with AGG having lower accuracy than AGA.

**Discussion**

In the current study, IgA tTG was found to compare well with IgA EMA and fared better than both AGA and AGG for the diagnosis of CD. Other authors have also reported the guinea pig IgA tTG to have similar sensitivity and specificity, and good concordance with EMA. In recent studies that used human (recombinant or red blood cell) tissue transglutaminase, the test performance has improved even further. Being an EIA test, IgA tTG also avoids inter-observer variations in interpretation, which are seen with EMA. In comparison, the anti-gliadin antibody tests fared poorly than EMA and tTG; in particular, AGG was the least specific of the four tests studied and had many false positives. Other workers too have shown the AGG to be positive in healthy subjects and in subjects with gastrointestinal inflammation due to various causes.

According to the modified ESPGHAN criteria, only intestinal biopsy changes and response to GFD are sufficient to make a diagnosis of CD. However, many conditions other than CD may be associated with villous atrophy; this is particularly true in developing countries. In a series of 121 children with protracted diarrhea from Delhi, Khosho et al showed that 32 children had severe villous atrophy due to diseases other than CD, including enteropathogenic *Escherichia coli* infection in 8 children, giardiasis in 8, cow’s milk protein intolerance in 5, salmonella in 4, bacterial overgrowth in 4, and transient gluten intolerance in three. In another report from India, 7 of 57 (12%) children suspected to have CD and having villous atrophy did not respond to GFD; the final diagnoses in these patients were cow’s milk protein intolerance and protein-energy malnutrition in two children each, and Crohn’s disease, bacterial overgrowth and giardiasis in one case each. Primary protein-energy malnutrition and recurrent gastrointestinal infections are also known to adversely affect small bowel histology. Therefore, in developing countries, villous atrophy is not always synonymous with CD. This fact may lead to a heightened role for serological tests in the diagnosis of CD in these regions.

Ideally, gluten challenge is essential for the diagnosis of CD. However, this is difficult, time-consuming, requires patient and parents’ cooperation, and is also not advisable during adolescence. The other option is therefore to test for CD serology. We found IgA tTG as a useful marker for the diagnosis of CD, with sensitivity and specificity rates comparable to those of IgA EMA. We suggest that serological tests like EMA or IgA tTG should be done at the time of diagnosis of CD; in the clinical setting of CD, presence of these antibodies along with typical small intestinal mucosal changes may increase the likelihood of the diagnosis of CD, even before response to GFD is available.

To conclude, we have shown that IgA tTG test compares favorably with IgA EMA, both for diagnosis of CD and its differentiation from other causes of diarrhea, while being better in ease of performance. Antigliadin antibodies do not perform as well and thus are not recommended.
References


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