Titters of Anti-tissue Transglutaminase Antibody Correlate Well With Severity of Villous Abnormalities in Celiac Disease

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Goals: We reviewed our celiac disease (CeD) database to study if anti-tissue transglutaminase (tTG) antibody (ab) titers correlate with severity of villous abnormalities in Indian patients and to find out a cutoff value of anti-tTG ab fold-rise, which could best predict CeD.

Background: Guidelines for diagnosing CeD suggest that biopsy could be avoided in some patients with high anti-tTG ab titer.

Study: We reviewed a cohort of 366 anti-tTG ab-positive individuals in whom duodenal biopsies were performed. Anti-tTG ab was obtained before initiation of gluten-free diet. Anti-tTG ab results were expressed in terms of fold-rise by calculating ratio of observed values with cutoff value. CeD was diagnosed if in addition to positive serology, patients had villous atrophy (>Marsh grade 2) and unequivocal response to gluten-free diet.

Results: The mean anti-tTG fold-rise in groups with Marsh grade ≤2 was 2.6 (± 2.5), grade 3a was 4.0 (± 3.9), 3b was 5.7 (± 5.1), and 3c was 11.8 (± 9.0). The positive likelihood ratio for diagnosing CeD was 15.4 and 27.4 at 12- and 14-fold-rise of anti-tTG ab titer, respectively. The positive predictive value of diagnosis of CeD was 100% when anti-tTG ab titer was 14-fold higher over the cutoff value. Fifty-seven (43.9%) individuals with anti-tTG ab titer <2-fold high also had CeD.

Conclusions: As severity of villous abnormality increases, titer of anti-tTG also rises. Presence of villous atrophy can be predicted at very high anti-tTG ab titer. In contrast to emerging belief, mucosal biopsies should be performed even if anti-tTG ab titer is <2 times, because many patients with CeD have low titers.

Key Words: serology, histology, villous atrophy, enteropathy (J Clin Gastroenterol 2014;00:000-000)

Celiac disease (CeD) is an autoimmune disease, which occurs in genetically susceptible individuals and affects 1% of the world’s population.1 The diagnosis of CeD has been based classically on a triad of clinical manifestations, demonstration of villous abnormalities of various severity (assessed by histologic evaluation of the small intestinal mucosa), and unequivocal response to prolonged and continued avoidance of the dietary antigen.2,3 Advent of celiac-specific antibodies (a reflection of adaptive immune response to gluten peptide) has revolutionized the case detection rate and has led to recognition of CeD as a public health problem world over. In the present time celiac-specific serological tests including anti-tissue transglutaminase antibody (anti-tTG ab), anti-endomysial antibodies (AEA), and anti-deamidated gliadin peptide have become an integral part of not only screening and diagnosis but also for follow-up of patients with CeD.1,2

Recently, many studies on pediatric patients with CeD have suggested that high anti-tTG ab titers are almost 100% predictive of presence of villous atrophy on mucosal biopsies.4–8 On the basis of these evidences, the recent guidelines by European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) have suggested that biopsies could be avoided in a small subset of patients who have very high titers of anti-tTG ab (>10 times upper limit of normal) in the presence of a positive anti-endomysial antibody and presence of susceptible HLA haplotype, that is, HLA-DQ2 and/or HLA-DQ8.2

The data on positive predictive values (PPV) of high anti-tTG ab titers for diagnosing CeD in adult population is limited.5–9 Furthermore, there is no data from tropical countries where there are many other causes of villous atrophy including tropical sprue, chronic giardiasis, protein energy malnutrition, and bacterial overgrowth.10 Thus, in these countries, celiac-specific serological tests play even more pivotal role in the diagnosis of CeD.

CeD is emerging in Asia and prevalence of CeD has been found to be similar to western countries in many Asian countries including India and Iran.11–13 As a large proportion of patients with CeD in these countries still remain undiagnosed, it will be interesting to see if similar correlation exists between anti-tTG ab titer and severity of villous abnormalities in Indian patients. Such data will help in making diagnosis of CeD much simpler in resource-limited countries, where there is a large pool of patients with CeD and disproportionately limited availabilities of gastroenterologists, endoscopic facilities, and trained pathologists. We, therefore, reviewed our data of patients with CeD to study if anti-tTG ab titer correlate with severity of villous abnormalities and to find out a cutoff value of anti-tTG fold-rise, which best predicts presence of CeD.

PATIENTS AND METHODS

In this retrospective analysis, we reviewed the database of individuals tested anti-tTG ab—positive at our center. These individuals either presented with clinical
manifestations of CeD to the outpatient clinic or were detected to be anti-tTG-positive during a population-based prevalence study. Individuals who had a positive anti-tTG ab and where biopsy blocks were available were included in this study. Any individual who was on gluten-restricted or gluten-free diet before serological and/or histologic tests were excluded from this study.

Over the period of time, the estimation of anti-tTG ab in our laboratory was carried out using > 1 ELISA kits procured from different manufacturers: the Binding Site Limited (Birmingham, UK), Inova Diagnostics (San Diego, CA), and AESKU Diagnostics (Wendelsheim, Germany). tTG tests were carried out as per the manufacturer’s instructions. Positive and negative controls were used. An anti-tTG titer of > 4 U/mL for Binding Site, > 20 U/mL for Inova diagnostics, and > 18 U/mL for AESKU Diagnostics was considered as positive for CeD. In a few patients, the serological tests were conducted from commercially available laboratories. The cutoff values for a positive test varied between different diagnostic ELISA kits. To make uniformity, the titer of anti-tTG ab was also estimated again in a few patients if serum was stored with us. The results of anti-tTG ab titer were expressed as folds rise above the cutoff value (by calculating the ratio of observed anti-tTG ab titer divided by cutoff value for a positive test). Serum samples for serological testing were obtained within a month before obtaining intestinal biopsies. A few patients had undergone serological testing at 2 or multiple occasions but had not undergone mucosal biopsy testing. In such patients, only the most recent serology test (within 1 month) before the mucosal biopsy was considered. The gap between serology testing and obtaining of biopsies was < 1 month in all the patients.

All these patients had undergone a standard diagnostic work-up as a standard of care at our center. All the patients had undergone esophagogastro-duodenoscopy and at least 4 to 6 biopsies had been obtained from the third part of the duodenum. A biopsy fragment was considered as oriented when at least 3 crypts were oriented perpendicularly on the underlying muscularis mucosae. Biopsies were analyzed for mucosal changes by 2 histopathologists with special interest in gastrointestinal pathology who were blinded to the clinical or serological results. The Modified Marsh grading system was used for grading mucosal changes: grade 0, normal histology (a crypt to villous ratio of 1:3 was taken as normal); grade 1: increase of intraepithelial lymphocytes (IEL) > 40/100 enterocytes (IELs were identified as dark round cells with high nucleus to cytoplasmic ratio, in comparison with the perpendicularly oriented cigar-shaped vesicular nuclei of the mucosal epithelial cells); grade 2: increased IELs along with crypt hyperplasia; and grade 3: increased IELs along with crypt hyperplasia and variable degrees of villous atrophy. Only lymphocytic infiltrate at the tip of the villi (crescendo pattern) was taken in account. Crypt hyperplasia was identified as enlarged tortuous crypts with basophilic nuclei. A further semiquantitative subtyping of the villous atrophies was performed as follows: grade 3a/mild villous atrophy (duodenal biopsies with a crypt to villous ratio of > 1:3 but < 1); grade 3b/moderate villous atrophy, C.V ratio of 1:1; grade 3c/severe villous atrophy, C.V ratio of > 1. IELs were further subclassified empirically as follows: 1 + , IELs > 40 but ≤ 60/100 enterocytes; 2 + , IELs > 60 but ≤ 80/100 enterocytes; and 3 + , IELs > 80/100 enterocytes. The type and density of inflammatory infiltrate and extent of edema in lamina propria was recorded.

The diagnosis of CeD was made on the basis of the modified European Society of Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) criteria, that is, clinical features, presence of villous atrophy (villous abnormalities of modified Marsh grade > 2), and unequivocal response to gluten-free diet. Chronic diarrhea was defined as diarrhea persisting for > 4 weeks. Anemia was diagnosed using WHO criteria for anemia, that is, < 13 g/dL in male patients and < 12 g/dL in female patients.

**Statistical Analysis**

Analysis was carried out on STATA software, version 12.1. P-value of < 0.05 was considered as significant. Anti-tTG ab fold-rise was expressed as mean ± SD and was compared between cases and control using the Student t test. Anti-tTG ab fold-rise was compared with various grades of villous atrophy using 1-way analysis of variance. ROC characteristics were determined to assess the discriminating ability of anti-tTG ab titer for presence or absence of villous atrophy. Sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and positive predictive value for the presence of diagnosis of CeD was calculated based on the titer of anti-tTG ab. Similar analysis was conducted on CeD patients who presented with or without diarrhea and anemia.

**RESULTS**

A total of 366 anti-tTG ab–positive (169 male and 197 female patients) individuals were included in this study. The mean age (± SD) of the patients was 28.9 ± 12.4 years. The median duration of symptoms before the diagnosis of CeD was made was 36 months (interquartile range, 12 to 72 mo).

**Mode of Presentation**

The most common mode of presentation was chronic or recurrent diarrhea (147 patients, 40.2%), short stature (51, 13.9%), and refractory anemia (40, 10.9%). Sixty-eight patients (18.6%) were detected to have an anti-tTG ab during a general population prevalence study.

**Grade of Villous Abnormalities**

In this cohort, the maximum number of patients (137, 37.4%) had villous abnormalities of modified Marsh grade 3c. The villous abnormality of modified Marsh grade 0 was present in 63 (17.2%), grade 1 in 14 (3.8%), grade 2 in 33 (9.1%), grade 3a in 63 (17.2%), and grade 3b in 56 individuals (15.3%).

**Correlation Between Titer of Anti-tTG ab and Severity of Villous Abnormalities**

The mean anti-tTG ab fold-rise in group of patients with modified Marsh grade ≤ 2 was 2.6 (± 2.5), grade 3a was 4 (± 3.9), grade 3b was 5.7 (± 5.2), and grade 3c was 11.8 (± 8.00) (Fig. 1). The mean anti-tTG ab fold-rise in patients with CeD was 8.7 (± 7.6). The mean anti-tTG fold-rise in group with grade 3c was significantly higher as compared with 3 other groups (P-value < 0.001 for each). Similarly, the mean tTG fold-rise in the group with grade 3b was significantly higher than those with grade ≤ 2 (P-value < 0.001).

There was no effect of duration of symptoms of CeD on the correlation between anti-tTG ab titers and severity.
Mean anti-transglutaminase (tTG) fold-rise in patients with celiac disease (CeD) having various grades of villous abnormalities.

of villous abnormalities. However, patients having villous abnormalities of Marsh grade 3c had significantly higher anti-tTG fold-rise, both in patients with duration of symptoms >12 months (115 patients) and those with symptoms ≤12 months (51 patients).

Correlation between degree of villous damage and anti-tTG titer were analyzed separately for both adolescent (≤18 y) as well as adult patients (>18 y). In both the groups, patients with severe villous abnormalities had significantly higher anti-tTG fold-rise as compared with those with villous abnormalities of modified Marsh grade 2, 3a, or 3b.

Assessment of a Positive Predictive Value for Anti-tTG Titer Fold-Rise

The PPV at various anti-tTG fold-rise levels for diagnosis of CeD has been shown in Table 1. As expected, the PPV for diagnosing CeD increased with progressive increase in the anti-tTG titer fold-rise. The prediction of CeD, irrespective of symptoms, was 100% if anti-tTG titer was 14-fold higher than the cutoff titer for a positive test. Furthermore, 57 (43.8%) individuals having low-titer–positive (<2-fold) anti-tTG ab also had CeD.

Likelihood Ratio for Diagnosis of CeD at Various Levels of Anti-tTG Titers

Positive likelihood ratio for diagnosing CeD increased with increase in anti-tTG fold-rise levels (Table 2). The positive likelihood ratio for diagnosing CeD was 15.4 and 28.7 at 12- and 14-fold-rise of anti-tTG titers, respectively. As expected, specificity also increased with increase in anti-tTG fold-rise and reached 100% at cutoff of 14-fold-rise.

Combination of Clinical Symptoms and Anti-tTG Titer on Prediction of Severity of Villous Abnormalities and CeD

Effect of Chronic Diarrhea on PPV for Diagnosing CeD

The data on presence or absence of chronic diarrhea were available in 338 patients. The PPV for diagnosing CeD was 100% at the anti-tTG titer >12-fold if the patient had diarrhea (Table 3), which was <14-fold-rise of anti-tTG required for 100% PPV for diagnosing CeD irrespective of symptoms. At each fold-rise of anti-tTG titer, the PPV for diagnosing CeD was higher for those who had diarrhea compared with those who did not have diarrhea.

Effect of Anemia on PPV for Diagnosing CeD

The data on hemoglobin was present in 313 patients. The PPV for diagnosing CeD was 100% at 14-fold-rise of anti-tTG titer in presence of anemia, which was same as anti-tTG fold-rise required for 100% PPV for diagnosing CeD in the overall study population. At each fold-rise of anti-tTG titer, the PPV for diagnosing CeD was higher for the anemia group when compared with group without anemia (Table 4).

Effect of Both Diarrhea and Anemia on PPV for Diagnosing CeD

A total of 147 patients had both diarrhea and anemia. The PPV for diagnosing CeD was 100% when the anti-tTG titer was ≥8.5-fold above cutoff values, which was much lower than tTG fold-rise required for 100% PPV for diagnosing CeD in the other groups mentioned above.

Correlation Between Presence of Anemia With Degree of Villous Atrophy and Anti-tTG Titer

The mean hemoglobin in patients having villous abnormalities of Marsh grade 3a, grade 3b, and grade 3c were 11.8 ± 2.2, 10.5 ± 2.7, 10.4 ± 2.6, and 8.8 ± 2.3 g/dL, respectively. The mean hemoglobin in group with severe villous atrophy (Marsh grade 3C) was significantly lower than the other 3 groups (P-value < 0.001 for each). Similarly, patients with mild and moderate villous atrophy had significantly higher mean hemoglobin levels when compared with those with no villous atrophy (P-value 0.008 and 0.01, respectively). Similarly, as the anti-tTG titer fold-rise increased in these patients, the mean hemoglobin levels decreased (P-value < 0.001 and r = −0.28) (Fig. 2).

DISCUSSION

In the present study, we demonstrated that as titer of anti-tTG ab rises, the severity of villous abnormalities also increases. The PPV was 100% for presence of villous atrophy of modified Marsh grade 3 if anti-tTG ab titer was >14-fold. Furthermore, in presence of symptoms such as diarrhea or anemia, the PPV for presence of villous atrophy was 100%, even at lower level of anti-tTG ab titer (12-fold

| Table 1. Positive Predictive Value for Diagnosing CeD at Various Anti-tTG ab Levels |

<table>
<thead>
<tr>
<th>Titer of Anti-tTG ab (Expressed as Fold-Rise)</th>
<th>Villous Abnormalities (Modified Marsh Grade &gt; 2)</th>
<th>Villous Abnormalities (Modified Marsh Grade ≤ 2)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 1 - &lt; 2</td>
<td>57</td>
<td>73</td>
<td>43.8</td>
</tr>
<tr>
<td>≥ 2</td>
<td>199</td>
<td>37</td>
<td>84.3</td>
</tr>
<tr>
<td>≥ 5</td>
<td>139</td>
<td>14</td>
<td>90.9</td>
</tr>
<tr>
<td>≥ 7</td>
<td>117</td>
<td>07</td>
<td>94.4</td>
</tr>
<tr>
<td>≥ 10</td>
<td>89</td>
<td>05</td>
<td>94.7</td>
</tr>
<tr>
<td>≥ 12</td>
<td>72</td>
<td>01</td>
<td>98.6</td>
</tr>
<tr>
<td>≥ 14</td>
<td>61</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

ab indicates antibody; CeD, celiac disease; PPV, positive predictive value; tTG, transglutaminase.
The presence of villous abnormalities

Several studies have suggested that the serological titers correlate well with degree of villous abnormalities. A multicentre study on 412 children by Alessio et al has shown that an anti-tTG titer fold-rise of ≥7 was able to identify all the children with significant mucosal damage (Marsh grade ≥2). Hill and Holmes reported a PPV of 100% for diagnosing CeD at an anti-tTG ab fold-rise of 10-fold in adult patients with CeD. These studies and data from the present study suggest that high titers of anti-tTG ab could be used as a surrogate marker of presence of significant villous abnormalities and for the diagnosis of CeD.

Not only a correlation exists between the titer of anti-tTG ab and severity of villous abnormalities, but a correlation between villous abnormalities and gastrointestinal symptoms, laboratory parameters, and health-related quality of life has also been shown by Taavela et al. A similar correlation was observed between anti-tTG ab titer and symptoms and laboratory parameters in the present study as well. We also observed lower mean hemoglobin with increase in the titer of anti-tTG ab. Similar correlation between hemoglobin level and severity of villous damage has recently been described in Indian children with CeD.

CeD is evolving all over the world, including certain Asian countries. The presence of villous abnormalities could be found in many other conditions such as tropical sprue, giardiasis, and small bowel bacterial overgrowth. Furthermore, there is an overlap between symptoms of tropical sprue and CeD, and it is extremely difficult to differentiate these 2 conditions based on clinical and histologic features. CeD-specific serological tests are of great help in differentiating these 2 conditions.

<table>
<thead>
<tr>
<th>Titer of Anti-tTG ab (Expressed as Fold-Rise)</th>
<th>Sensitivity for Villous Abnormality (Modified Marsh Grade &gt; 2) (%)</th>
<th>Specificity for Villous Abnormality (Modified Marsh Grade &gt; 2) (%)</th>
<th>Positive Likelihood Ratio for Villous Abnormality (Modified Marsh Grade &gt; 2)</th>
<th>Negative Likelihood Ratio for Villous Abnormality (Modified Marsh Grade &gt; 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 14</td>
<td>24.2</td>
<td>100</td>
<td>28.7</td>
<td>0.8</td>
</tr>
<tr>
<td>≥ 12</td>
<td>28.1</td>
<td>98.2</td>
<td>15.4</td>
<td>0.7</td>
</tr>
<tr>
<td>≥ 10</td>
<td>34.8</td>
<td>95.5</td>
<td>7.6</td>
<td>0.7</td>
</tr>
<tr>
<td>≥ 5</td>
<td>46.1</td>
<td>93.6</td>
<td>7.2</td>
<td>0.6</td>
</tr>
<tr>
<td>≥ 2</td>
<td>54.3</td>
<td>87.3</td>
<td>4.3</td>
<td>0.5</td>
</tr>
<tr>
<td>≥ 1</td>
<td>77.7</td>
<td>66.4</td>
<td>2.3</td>
<td>0.3</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>1− &lt; 2</td>
<td>45.3</td>
<td>93.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

ab indicates antibody; CeD, celiac disease; tTG, transglutaminase.

TABLE 3. Effect of Clinical Manifestation (Chronic Diarrhea) on PPV of Anti-tTG ab Fold-Rise Levels for Diagnosis of CeD

<table>
<thead>
<tr>
<th>Fold-Rise in Anti-tTG ab</th>
<th>Patients Having Chronic Diarrhea (n = 204)</th>
<th>Patients Without Chronic Diarrhea (n = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CeD</td>
<td>No CeD</td>
</tr>
<tr>
<td>≥ 1− &lt; 2</td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td>≥ 2</td>
<td>131</td>
<td>09</td>
</tr>
<tr>
<td>≥ 5</td>
<td>96</td>
<td>03</td>
</tr>
<tr>
<td>≥ 7</td>
<td>83</td>
<td>03</td>
</tr>
<tr>
<td>≥ 10</td>
<td>63</td>
<td>01</td>
</tr>
<tr>
<td>≥ 12</td>
<td>50</td>
<td>01</td>
</tr>
<tr>
<td>≥ 14</td>
<td>44</td>
<td>01</td>
</tr>
</tbody>
</table>

ab indicates antibody; CeD, celiac disease; PPV, positive predictive value; tTG, transglutaminase.
What could be the biologic basis of the correlation between severity of villous abnormalities and the titer of anti-tTG ab? There are more opinions than the facts. Taking an analogy, a correlation has been found between anti-dsDNA antibody level and the disease activity of systemic lupus erythematosus. Furthermore, anti-dsDNA ab has also been shown to be involved in the pathogenesis of systemic lupus erythematosus. In fact, anti-tTG ab have been implicated in the pathogenesis of CED. In vitro study using T84 crypt cell fibroblast coculture, Halttunen and Maki observed significant inhibition of T84 epithelial cell differentiation and increase in epithelial cell proliferation with addition of monoclonal antibodies against tTG. The authors speculated that this effect was caused by the blockage of the activating action displayed by tTG on TGF-β. In fact, TGF-β is normally secreted in a latent form and needs to be activated for biological activity, and tTG is known to be involved in the activation of latent TGF-β.

The present study, a large number of patients (n = 110) having early villous changes (modified Marsh grade < 3) were included, which is one of the merits. In conclusion, as the severity of villous abnormality increases, the titer of anti-tTG ab also increases. Duodenal biopsy could be avoided in some individuals with very high anti-tTG ab titer (>14 times). In contrast to emerging belief, mucosal biopsies should be carried out even in those with anti-tTG titer < 2-fold-rise.

### REFERENCES


