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Original Article

Correlation of serum levels of IgA anti-tissue transglutaminase (IgA tTG) with the histological severity in celiac disease

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ABSTRACT

Background: With high diagnostic accuracy of serological test like Ig A tissue transglutaminase (tTG) in celiac disease (CD), the necessity for small intestinal biopsy has been questioned.

Aim: To ascertain the correlation between serum levels of IgA anti- tTG with histological severity and to predict the cut-off tTG levels, which would predict the presence of Marsh ≥2 changes in histology diagnostic of CD.

Methods: A prospective study was done in the pediatric age group of 2-18 years with suspected coeliac disease. All were tested for a-tTG, followed by endoscopic biopsy in patients with positive serology. Histology was assessed according to Modified Marsh grading. Receiver operating curve (ROC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), were used to find the cutoff anti-tTG levels confirming CD.

Results: 162 symptomatic subjects were included in the study of which 126 had histology confirmatory of CD.Marsh grade 2 changes were seen in 2.3%, 3a in 27%, 3b in 24.6% and 3c in 46 % respectively. Higher grades of Marsh injury were associated with progressively increasing anti tTG levels, which was statistically significant (p=0.015). ROC curve showed cutoff of 76 U/ml with sensitivity, specificity, PPV and NPV of 84.1%, 97.2%, 99.07% and 63.64 % respectively.

Conclusion: There is positive correlation between serum levels of anti tissue trans glutaminase and stage of mucosal injury, so the diagnosis of CD can be reached without endoscopic biopsy. This will avoid an invasive, traumatic and costly procedure in children and lead to more rapid diagnosis.

KEYWORDS: IgA anti tissue transglutaminase antibody; celiac disease; marsh grading; villous atrophy.

Introduction

Celiac disease (CD) is an immune-mediated enteropathy caused by permanent sensitivity to gluten in a genetically susceptible individuals.[1] Initially it was considered a malabsorption syndrome of childhood but is now recognized as a common condition that can occur at any age. The overall prevalence of celiac disease in North India is 1.04%.[2] Clinical manifestations vary greatly, classical CD presents with diarrhea, steatorrhoea, weight loss, while non-classical CD with anemia, osteopenia, osteoporosis, recurrent abortions, hepatic steatosis, elevated liver enzymes and recurrent aphthous stomatitis.[3] Recent evidences have debated the practice of carrying out duodenal biopsies in all patients testing positive for anti-tissue trans glutaminase (tTG) antibodies and an hypothesis has been put forward that biopsy may be avoided in individuals with very high titers of serum antibody levels.[4-7] On the similar lines European[8] Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines published in 2012 have suggested that the diagnosis of CD can be established without small intestine biopsy in genetically pre-disposed children who are symptomatic and have anti-tTG levels 10 times greater than the upper limit of normal (ULN), positive EMA and good response to a gluten-free diet (GFD). However, there is paucity of studies from India, for correlating serum antibody levels with histological damage and as well to define the cut-off anti-tTG value that would predict CD. A prospective study by Bhattacharya[9] et al, in children less than 12 years, has shown that with anti-tTG titer of 62.5 U/mL (four times upper limit of normal) the positive predictive value (PPV) of 93.8% for predicting CD. Another recent study has shown that the prediction of CD, irrespective of symptoms was 100% if anti-tTG titer was 14-fold higher than the cutoff titer for a positive test.

The aim of present study is to find, if anti tTG antibody correlate with histological severity and to establish the cut-off values of anti -tTG that would predict the presence of duodenal histology (Marsh ≥ 2) diagnostic for CD.

Methods

It was a single center prospective observational study, from January 2014 to Feburary 2015. During the study period all symptomatic (both typical and atypical symptoms) patients between the age group of 2 to 18 years attending gastroenterology out patient department and referred for endoscopic biopsy for evaluation of celiac disease were enrolled. Children less than 2 years of age, those already on gluten free diet (GFD) and negative serology (<18U/ml) were excluded. The demographic profile, associated factors for like family history of CD, diabetes mellitus, thyroid disorders and chronic liver disease (CLD) and biochemical parameters were noted. Ethics committee clearance was obtained for the study from the institute.

Serology

All children in the cohort underwent serum anti-tTG levels measured centrally at our institution. Estimation of serum anti-tTG was done by recombinant human ELISA kit (CHORUS tTg-A, 86058, Siena, Italy). Antibody level >18 U/ml were considered as positive as per manufacturer recommendation and it had calibration range of 3 to 100 U/ml. Sample with titers >100 U/ml were retested to confirm the results.

Duodenal Biopsy

Upper gastrointestinal endoscopy was carried out in patients with positive serology (tTG>18 U/ml), after obtaining written informed consent from the caretakers. Minimum of 4 duodenum biopsies were taken, specimen was oriented and are reviewed by the pathologist at our institution, who was blinded to serum tTG titers. The histological severity was documented as per the modified Marsh classification, i.e. 0, normal; 1, increased intraepithelial lymphocytes; 2, increased intraepithelial lymphocytes and crypt hyperplasia; 3a, partial villous atrophy; 3b, subtotal villous atrophy; and 3c, complete villous atrophy.[11] Diagnosis of CD was established on marsh grade of ≥ 2 with positive serology and was started on gluten free diet.

Statistical Analysis

The data was analyzed using computer statistical software (Microsoft Excel, SPSS 20). Descriptive statistics (mean, standard deviation and proportions) were used to summarize the variables. Student's t-test for independent samples was used to compare the mean values of continuous variables. ANOVA (analysis of variance) test was used to observe an association between anti-tTG and Marsh grading. Receiver operated curve (ROC) curve was used to determine the best cut-off for anti-TTG value with best sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV), that predict presence of Marsh grade ≥ 2 histology indicative of CD. Pearson's coefficient correlation was plotted to assess the association of hemoglobin with tTG titers. A p value of less than 0.05 was considered statistically significant.

Results

162 patients were enrolled during the study period, of which 126 had ≥2 Marsh changes in duodenal histology suggestive of CD, while 36 had early (0 and 1) changes and were considered as non CD. Out of 126 CD patients, 51 (40.4%) were males and 75 (59.5%) were females, with a male to female ratio of 0.68:1. Their mean age was 8.2±4.43 years and mean duration of symptoms at presentation was 21.7±10.8 months. These subjects presented with diarrhea 52.4%, anemia 49.1%, failure to thrive 42.2%, short stature 38.8%, pain abdomen 31.7%, abdominal bloating 23%, vomiting 12.6%, constipation 10.2%, weight loss 8.2%, or at risk of CD (positive family history 15.2%, thyroid disorders 12.9%, chronic liver disease 12.1% and diabetes 7.7%). Biochemical investigation revealed hemoglobin 9.6±1.7 (gram/dl, mean ± standard deviation), platelet count 2.96±1.1 (normal 1.5-4.5 lakhs/mm^3), mean corpuscular volume (MCV) 71±10.57, aspartate transaminase (AST) 49.3±20.57, (normal 0-50 IU/l), alanine transaminase(ALT), 44.6±18.29(0-40 IU/L).

Correlation of anti-tTG titres with histological severity

Marsh grade 2 changes were seen in 3 (2.3%), 3a in 34 (27%), 3b in 31 (24.6%) and 3c in 58 (46%) of patients respectively. A higher grade of Marsh injury was associated with progressively increasing anti-tTG antibody titers, which was statistically significant (p=<0.015). (**Table 1**).

When exploring the accuracy of anti-tTG levels for diagnosing CD, the best cut-off value of a-tTG in predicting Marsh ≥ 2 at histology was found to be 76 U/mL, with sensitivity, specificity, PPV and NPV of 84.1%, 97.2%, 99.07% and 63.64% respectively, (**Figure 1**). Receiver operating characteristic (ROC) curve showed area under the curve of (AUC)=0.94, {with 95% confidence interval (0.94 to 0.97)}, at a cut-off of 76 U/ml, which corresponds to four times the upper limit of normal of our kit. (**Figure 1**).

Association of hemoglobin with anti-tTGtitres and histological severity

We also noted that patients with biopsy suggestive of CD (Marsh ≥2) had lower mean hemoglobin in contrast to patients with negative biopsy (Marsh 0 &1changes), (Mean±SD, 9.61±1.68 vs 11.64±1.54) which was statistically significant (p<0.001).

Similarly asignificant inverse relationship was also noted between hemoglobin and raising tTG titers, with Pearson's co-efficient correlation of (r=-0.278, p=0.002). (**Figure 2**).

Table 1: Association of marsh grading with anti- IgA TTG titres

MARSH GRADE	n =126,	anti-tTG titers in U/ml (Mean+SD)	ANOVA TEST
2	3	72.00 ± 25.87	0.015
3a	34	83.21 ± 25.76	
3b	31	90.45 ± 16.50	
3c	58	94.28 ± 12.72	

Discussion

Duodenal histology is considered as the criterion standard for diagnosis, however, it is not specific for celiac disease, because other clinicaldisorders can show similar microscopic picture.[12] In addition, endoscopy/histology have disadvantages such as invasiveness, high costs, artifacts due to a non-longitudinal cut, poor specificity (especially for duodenal lymphocytosis).[13] Results of our study were comparable to other Indian studies. A prospective study of 142 patients from Delhi[8] in children showed similar cutoff anti -tTG levels (four times in both), in contrast our results had better PPV (99% vs 93.8%). Another retrospective study of 366 patients[9] from AIIMS Delhi showed higher cutoff (14 times), irrespective of symptoms, but our study included only symptomatic subjects.

Other researchers also have showed similar correlation but with variable cut-offs. Barker[4] et al, in a retrospective evaluation on 103 children showed 48 of 49 patients with anti-TTG >100 U/mL (5 times the cutoff) had histological diagnosis of CD (Marsh ≥2). Sensitivity and specificity were 98% and 97.2%, respectively. Vivas et al[5], in a study of 324 patients including both adult and children showed with the cut-off point of 30 U tTG antibody (10 times the normal) yielded sensitivity and specificity of 98% & 98% respectively.

Donaldson[6] et al demonstrated with an anti tTG value of 40 u/ml (twicethe upper limit of normal) had sensitivity, specificity, PPV, and NPV of 82.1%, 98.4%, 97.9%, and 85.7%, respectively, for Marsh 3a on 177 celiac pediatric patients.Tortora et al[7], in a prospective study showed the best cut-off value of anti tTG for predicting Marsh ≥2 was 45 U/mL (6.4 times upper limit of normal) with sensitivity 70%; specificity 100%; PPV 100%; NPV 24.1%, respectively. All these authors concluded that endoscopy and biopsy could be avoided in subset of patients with high serum titers of anti-tTG levels.

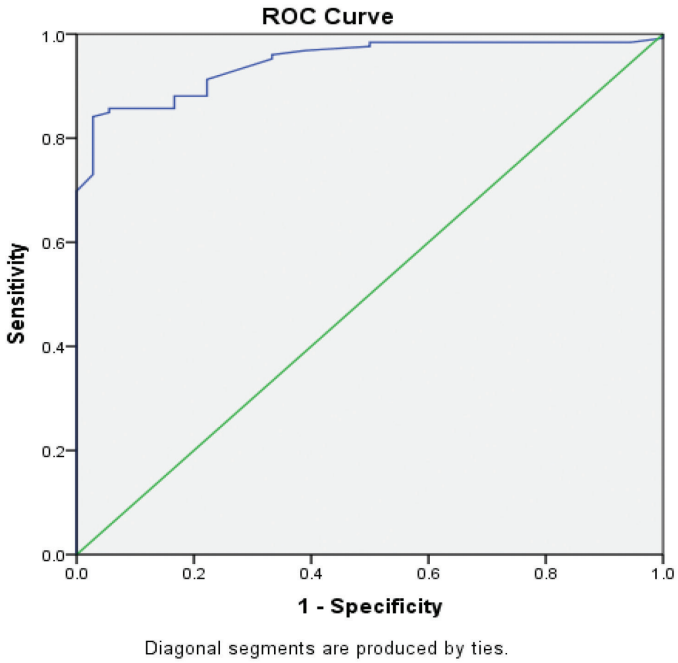

Figure 1: ROC showing maximum area under the curve for marsh (≥2) grade in biopsy suggestive of celiac disease at tTG titre of 76 u/ml.

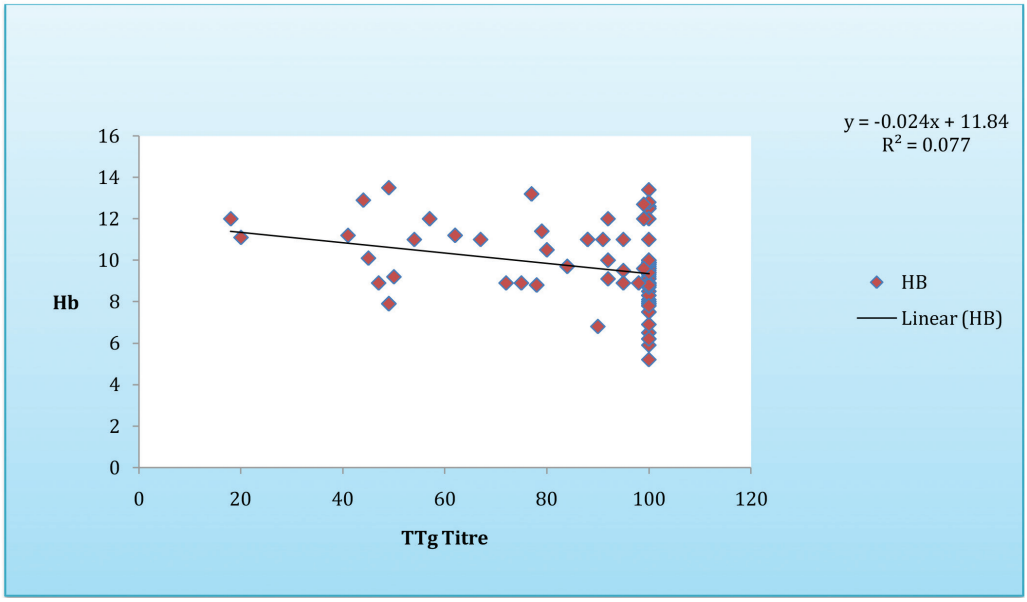

Figure 2: Correlation between IgA tTG titre and hemoglobin by Pearson's correlation coefficient.

An inverse correlation of hemoglobin was noted with Marsh grades and higher tTG levels, indicating increasing severity of anemia with progressive destruction of mucosal epithelium. Previous researchers in India also noted similar findings.[10,14] Addition of hemoglobin levels might increase the confidence for diagnosis of CD, but optimal levels need to be determined. CD is a much greater problem in India[2] than that has been previously thought. The prevalence of CD in Indian community is as same as that reported from west. Growing awareness of disease across physicians, screening serological tests for CD are done in most peripheral centers but specialists to perform pediatric endoscopy are not widely available, which is increasing the burden on family members.

There are problems with tTG assays. The values for serum anti-tTG obtained in a test depend on the source (human or animal) of antigen, measuring methods, cutoff values and calculation mode of the results, so numerical values obtained with different kits may differ substantially. Despite these differences, many commercial anti-tTG antibody tests have equally high sensitivity and specificity on the same blood samples.[15]

Limitations of the study include non-evaluation of anti endomysialantibody (EMA) and human leucocyte antigen (HLA) in our cohort of patients who had lesser grades of Marsh injury (0&1) also serum IgA levels were not measured.

Conclusion

Our study demonstrates that children who are symptomatic between 2 to 18 years of age and raised anti-tTG antibodies are associated with high likelihood of severe duodenal damage. Diagnosis of CD could be reached without endoscopic biopsy in subjects with anti-tTG levels of >76 U/mL (four times the upper limit of normal), while it remains essential tools in subjects with anti-tTG levels less than that. However large studies are required to establish the optimal cutoff levels for anti-tTG antibodies.

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