

SEROLOGICAL VERSUS HISTOLOGICAL DIAGNOSIS IN PEDIATRIC CELIAC DISEASE: IS THERE A NEED FOR SMALL BOWEL BIOPSY?

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ABSTRACT

Objective: To evaluate the role of tissue transglutaminase IgA antibody (TTG IgA Ab) in diagnosis of pediatric celiac disease (CD) taking small bowel biopsy as gold standard.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Pediatric Gastroenterology, the Children's Hospital & the Institute of Child Health, Lahore, from Jan 2018 to Jun 2018.

Methodology: Sixty patients aged 2-18 years, with suspicion of CD, having at least 3 presenting features from chronic diarrhea, malnutrition, short stature, anemia, abdominal distention and digital clubbing, were included. TTG IgA Ab titre, small bowel biopsy (SBB) and histopathology were done in all cases.

Results: Of the 60 participants, 22 (36.7%) were male and 38 (63.3%) were female with mean age of 6.56 ± 3.78 years. The calculated sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy are 94.1%, 96.1%, 96.9%, 92.5% & 95% respectively. TTG IgA Ab value more than 90 U/ml corresponds to Marsh 3b or higher grade lesion with 94.73% positive predictive value.

Conclusion: There is a strong correlation between TTG titres and degree of duodenal damage in patients suspected of CD. Biopsy can be avoided when TTG level is more than 9 times the manufacturer's cut off value.

Keywords: Pediatric celiac disease, Tissue transglutaminase IgA, Small bowel biopsy.

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INTRODUCTION

Celiac disease (CD) is a chronic, immune mediated enteropathy occurring in genetically predisposed individuals on exposure to dietary gluten. It is characterized by specific autoantibodies against tissue transglutaminase (TTG), endomysium, and / or deamidated gliadin peptide¹.

The incidence of the childhood CD is increasing continuously in the west probably due to very effective screening serological tests². The reported prevalence rates in children vary from 1% to 8.8% in various populations³. Most of the patients are asymptomatic therefore detection of affected patients is less than 10%¹. As wheat is the major staple food in Pakistan, contributing 72% of our daily caloric intake with estimates of around 124 kg per capita wheat consumption per year, CD can be considered as important health problem in our country⁴. Malabsorption occurs as a

result of damage to small intestinal villi, leading to chronic diarrhea and failure to thrive. Non specific symptoms include abdominal pain, osteoporosis, elevated transaminases and neurological symptoms. The symptoms of CD resolve once the gluten is withdrawn from the diet⁵. If left untreated, CD is associated with high rates of morbidity and mortality⁶.

The gold standard for diagnosing CD is a small bowel biopsy (SBB) which may show villous atrophy, crypt hyperplasia or intraepithelial lymphocytes, graded according to Modified Marsh Classification⁷. However the procedure is invasive, requires anesthesia/or sedation and has its associated risks. It may also miss diagnosis in some as there may be patchy involvement of small bowel⁸. Initial work up in patients with suspicion of CD includes TTG IgA Antibody (Ab) and anti endomyseal antibody (EMA). Currently testing for TTG IgA Ab is considered the best initial screening test⁹. Serologic tests are easy to perform, widely available, relatively cheap correlate well with severity of the disease and their

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sensitivity and specificity has increased in recent years. Drawbacks of these tests include a high false positive rate in patients with other autoimmune conditions.

Whether antibody tests can replace intestinal biopsies as the gold standard, is a long debate. European Society for Pediatric Gastroenterology, Hepatology & Nutrition (ESPGHAN) has given recommendations minus small bowel biopsies in symptomatic CD patients with very high IgA TTG titres (TTG Ab >10 X the upper limit of normal) plus confirmation with IgA-EMA and HLA typing⁹. ESPGHAN recommendations along with other papers suggest that conclusive serological evaluation may negate the need for biopsy¹⁰.

Until now the prospective trials for evaluation of antibody tests have been performed mainly in adults with only two prospective studies could be found in children^{11,12}. In one study all the patients had CD so the predictive values could not be calculated. In the second study the 10×ULN (upper limit of normal) rule did not lead to any false positive result. The objective of this study was to evaluate the role of TTG IgA Ab in diagnosis of CD taking small bowel biopsy as gold standard with the intent that the need for endoscopic biopsy may be obviated in a symptomatic child suspected of CD.

METHODOLOGY

This cross sectional survey was conducted at the department of Pediatric Gastroenterology, the Children's Hospital & the Institute of Child Health, Lahore, from January till June 2018. Employing purposive sampling technique, sample size of 60 was calculated taking prevalence of celiac disease as 2.5%, sensitivity as 96.1% specificity as 99.8%, desired precision 0.04 and confidence interval of 95% by using following formula^{13,14}.

$$n = \frac{(Z)^2 P(1 - P)}{e^2}$$

Z = Confidence Interval

P = Incidence

e = Desired precision

Pediatric patients aged 2-18 years with suspected CD, on gluten diet were included in the study after taking written informed consent from the parents, if they had 3 or more clinical features of any of the following,

- Chronic diarrhea: Loose stools lasting more than 4 weeks.
- Malnutrition: Weight for age less than -2 standard deviations (SD) of the WHO Child Growth Standards median.
- Short stature: Height-for-age less than -2 standard deviations below the WHO Child Growth Standards median.
- Anemia: Hemoglobin concentration less than 10G/dl.
- Abdominal distention.
- Digital clubbing.

Patients on gluten free diet or who already have been diagnosed with celiac disease, patients with chronic systemic diseases, patients having chronic diarrhea due to other causes e.g. chronic giardiasis, cystic fibrosis, abdominal tuberculosis and patients with autoimmune diseases e.g. diabetes mellitus, inflammatory bowel disease were excluded from the study. Three ml of venous blood was drawn; coagulated blood sample was subjected to analysis for TTG IgA along with serum IgA from the central laboratory of the Children's hospital, Lahore using an enzyme linked immunosorbent assay (ELISA) technique by a commercially available kit (ORG540 A, ORGENTEC Diagnostika GmbH, Germany). Antibody levels above 10U/ml were considered positive as per the manufacturer's recommended TTG IgA cut-off value. IgA deficient subjects were not included in the study. All reports were verified by the same consultant pathologist. Upper GI endoscopy and small bowel biopsy of all the patients was performed by a consultant gastroenterologist at endoscopy suite, biopsies were analyzed by consultant histopathologist at the Children's hospital, Lahore the Children's hospital, Lahore. The histopathology was classified as normal or according to modified Marsh

Criteria 1-3. CD was defined as a single positive biopsy with characteristic Marsh 3 findings.

Children satisfying the inclusion and exclusion criteria were included in the study. After taking consent age, gender, weight and other important features were recorded through a pre designed proforma. The data was analyzed using SPSS-20. Frequencies and percentages were calculated for the different qualitative variables while mean and standard deviation were calculated for quantitative variables. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the TTG IgA Ab were calculated as a tool to detect cases of celiac disease.

RESULTS

The study population included 22 (36.7%) male and 38 (63.3%) females. The age of the patients ranged from 2-15 years with a mean of 6.55 ± 3.78 years. The demographic characteristic of the participants is shown in table-I.

Table-I: Demographic features of study participants in celiac disease (#60).

	n (%)
Gender	
Male	22 (36.7%)
Female	38 (63.3%)
Characteristic	Study Participants
Age (Years)	6.56 ± 3.78
Chronic Diarrhea n (%)	47 (78.3%)
Malnutrition n (%)	49 (81.7%)
Short Stature n (%)	46 (76.7%)
Anemia n (%)	43 (71.7%)
Abdominal Distention n (%)	21 (35%)
Digital Clubbing n (%)	8 (13.3%)
TTG IgA Ab (U/ml) mean \pm SD	53.35 ± 61.26

Table-II: 2x2 contingency table in celiac disease children (#60).

	Positive Biopsy n=34	Negative Biopsy n=26	
Positive TTG IgA Ab	32	1	Positive predictive value 96.97%
Negative TTG IgA Ab	2	25	Negative predictive value 92.59%
	Sensitivity 94.12%	Specificity 96.15%	

TTG IgA Abs were positive (>10 U/ml) in 33 (55%) children and negative in 27 (45%) children. Histopathology revealed 34 (56.7%) children

suggestive of celiac disease with 32 true positive, 2 false negative while 26 (43.3%) children had negative biopsy with 25 true negative and 1 false positive. The calculated sensitivity, specificity, positive predictive value and negative predictive value are 94.12%, 96.12%, 96.97% & 92.59% respectively as shown in table-II. Diagnostic accuracy of TTGs IgA Ab was calculated as 95%. Among all the TTG positive patients, 19 patients had TTG value >90 U/ml. Eighteen of these patients were true positive with histopathological changes corresponding to Marsh 3b or higher grade lesion with 94.73% positive predictive value. This correlation of increased specificity with increasing TTGs is also depicted by the ROC curve as shown below.

DISCUSSION

Antibody tests are the initial tools used for screening of pediatric patients with suspected CD. They also help in deciding further investigations to diagnose or exclude CD. Systemic reviews have concluded that human recombinant TTG IgA Ab is the preferred test than the anti endomysial antibodies, for excluding celiac disease in symptomatic patients, and for screening of asymptomatic individuals¹⁵. When compared with deamidated gliadin peptide antibody test, TTG IgA Ab remains preferred test, for diagnosis and/or exclusion of celiac disease¹⁶. The deamidated gliadin peptides have shown to be useful in monitoring the compliance of gluten free diet¹⁷. HLA genotyping with a high diagnostic sensitivity but low specificity and has a primary role in excluding CD. Taking into account all these fac-

tors there is a growing debate over omission of performing SBB for confirmation of the diagnosis of CD in patients with high TTG IgA Ab levels.

The participating children were divided into CD positives and negatives according to the biopsy results, which are considered the gold standard for diagnosis of CD. Prevalence of CD in our study population was 56.7%, correlating closely with the results of Aziz *et al*¹³. The children included in this study were at high risk for developing CD due to the clinical symptoms and do not rep-

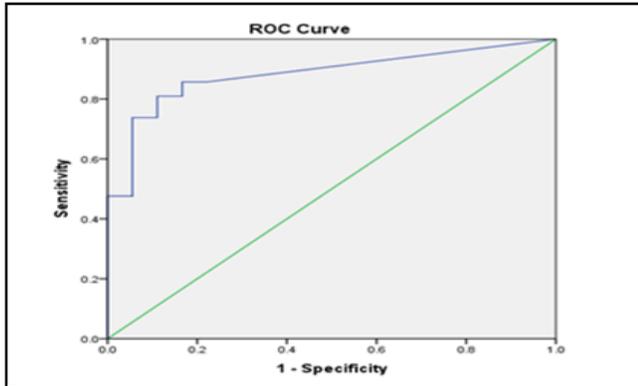


Figure: Diagonal segments are produced by ties.

resent the general pediatric population in our country. About 63.3% of our participants were females while 36.7% were males. Similar female preponderance has been reported by Shomaf *et al*¹⁸. Though a number of studies, mostly from developed countries, have reported towards a milder clinical picture of celiac disease^{19,20}, majority of our cohort presented with symptoms of classical CD. Our findings are supported by Rabhani *et al*²¹. A striking 71.7% of our patients had anemia similar to Jora *et al* who narrated the prevalence of anemia as 73% of their study population²². We recorded the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of TTG IgA Abs as 94.1%, 96.1%, 96.9%, 92.5% and 95% respectively. Alessio *et al* and Dean have demonstrated identical results in their studies^{14,23}.

A correlation between TTG IgA Ab and degrees of duodenal damage has been noted in our study. Levels more than 9 times or more above the manufacturer's cut off value (10 U/ml) i.e., TTG ≥ 90 U/ml was about 94.7% associated with histological lesion of Marsh 3b or higher. Rahmati *et al* used the similar ELISA technique and recor-

ded similar results⁷. Jora *et al* also concluded that the TTGs IgA Ab levels 9 times the cut off value can be used to predict villous atrophy with sensitivity of 100% and specificity of 85.7%²². Onyea-dor and fellows showed that children having TTG IgA Ab levels more than 10 times the ULN (>100 U/ml) correctly diagnosed CD in 98.3% of their cohort²⁴. Allesio and colleagues have documented high probability of duodenal damage in patients with positive TTG IgA Ab ≥ 7 times the cut off value¹⁴. Similar findings have been shown by Hashmi *et al*, who have demonstrated TTG IgA Ab >50 U/ml was associated with villous atrophy and histological lesion of Marsh 3b or high grade with positive predictive value of 100%²⁵.

In the light of the positive association between Abs levels and marsh grading, we propose that high titres of TTG IgA Abs (>90 U/ml) would be enough for CD diagnosis in symptomatic patients, eliminating the need for SBB. Since different kits use different cut off points and there is no standardization, our proposal should be examined with caution regarding other TTG IgA assays. Our data confirms that for high risk children, strong Ab levels could predict villous atrophy (Marsh 3b or higher) with high sensitivity 94.7%. These results are in agreement with recently published pediatric studies^{7,14,22,25}. In a resource constrained society like ours, overburdened by larger number of suspected CD patients and lack of costly equipment and trained personnel, TTGs IgA Abs ≥ 90 U/ml should be given consideration for gluten free trial. Patients should be followed up for improvement in clinical symptoms with repeat TTG levels after 6 weeks or so. Endoscopy and SBB should be reserved for TTG negative patients with strong clinical suspicion of CD and for cases with TTG positivity <90 U/ml.

The limitation of our study was lack of the control group and small size of our cohort. The diagnostic value of TTG IgA Ab could not be enhanced in our patients with anti endomyseal antibody or HLA analysis as these were not available at our hospital. Validation of our finding

needs a larger multicenter prospective trial, to conform its generalizability.

CONCLUSION

There is a strong correlation between TTG IgA Ab titres and degree of duodenal damage in patients suspected of CD. Biopsy can be avoided when TTG IgA Ab level is >9 times the manufacturer's cut off value. However, SBB should always be considered in case of high clinical suspicion regardless of the serological values.

CONFLICT OF INTEREST

The study has no conflict of interest to declare by any author.

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