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ORIGINAL ARTICLE

Accuracy of Anti-tissue Transglutaminase (TTG) Antibodies to Diagnose Celiac Disease in Children

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ABSTRACT

Objective: To determine the diagnostic accuracy of anti-tissue transglutaminase antibodies (TTG IgA) as a tool to detect celiac disease in patient presenting with clinical features suggestive of the disease keeping histopathology as a confirmatory test for diagnosis.

Study Design: Cross sectional study

Place and Duration of the Study: The study was carried out at department of pediatrics, Children's Hospital Lahore from 1st January to 30th June 2012.

Material and Methods: A total of 200 cases were enrolled after a written consent from parents/guardians. Data was recorded on proforma by researcher and then small bowel biopsy was performed. Patients were labeled as positive or negative for celiac disease based on TTG IgA and histopathology. Data was analyzed by using SPSS version-10. Statistical analysis was preformed to calculate means and proportions.

Results: In our study, out of 200 cases, mean and standard deviation for age was calculated as 6.74 $\pm$ 3.25 years, 59% (n=118) were male and 41% (n=82) were female, frequency of celiac disease was calculated as 20.5% (n=41). Keeping histopathology as gold standard, diagnostic accuracy of TTG IgA in detecting CD was calculated which showed sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate as 70.73%, 69.81%, 37.66%, 90.24% and 70% respectively.

Conclusion: We concluded that accuracy of TTG IgA in detection of CD in patients suspected for celiac disease is in acceptable range and this tool may be adopted for primary investigation of the morbidity. However, further trials are required to validate our findings.

Key Words: *Celiac Disease (CD), Anti-tissue transglutaminase antibodies (TTG IgA), Detection, Diagnostic accuracy*

INTRODUCTION

Celiac disease (CD) is an autoimmune condition characterized by a specific serological and histological profile triggered by gluten ingestion in genetically predisposed individuals.[1] Gluten is the general term for alcohol soluble proteins present in various cereals, including wheat, rye, barley, spelt, and kamut.[1] Celiac disease is primarily a digestive tract disease, it can cause complications in most organs of the body.[2] Epidemiologic studies report a global increase in the prevalence of the celiac disease, with different distribution patterns.

For example, in a study in Europe, its prevalence was high in Finland, but lower in Germany and Italy (0.3% and 0.7%, respectively).[2] CD is a chronic disorder which is characterized by inflammation and villous atrophy (VA) in the small intestine that affects people who are genetically predisposed.[3] Even though the prevalence of CD varies from region to region, the average prevalence of the disease has been reported between 0.5 and 1% worldwide.[4] Evidence suggests that CD is higher in patients with genetic and autoimmune diseases than in healthy individuals. Prevalence of CD is high in patients with insulin dependent diabetes mellitus type 1 (DM1), chronic diarrhea, autoimmune thyroid disease (ATD), autoimmune hepatitis, Down syndrome (DS), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), Turner syndrome (TS), and first-degree relatives (FDR) of patients with CD.[5,6] CD is a common cause of diarrhea, failure to thrive, abdominal distension, anemia, weight loss and short stature.[7] In India 16.6% children presenting with chronic diarrhea were diagnosed with CD in tertiary care hospitals.[8] Previously CD was diagnosed with frequent proximal intestinal biopsies and non-sophisticated serological investigations like anti gliadin and anti-endomysial antibodies which were complicated. Now as diagnostic facilities have improved, the criteria have been revised with sophisticated serological investigations including TTG IgA antibodies followed by gold standard proximal intestinal biopsy.[9] Human tissue transglutaminase is more accurate as antigen for specific determination of TTG IgA antibodies.[10] Antitissue transglutaminase antibodies are cheaper and simpler, more sensitive for mass screening than anti-endomysial antibodies assay.[11] The sensitivity and specificity of TTG IgA Enzyme-Linked Immunosorbent Assay (ELISA) is 90% and 95% respectively.[12] There are some conflicting results on tissue transglutaminase antibodies diagnostic strength. The overall sensitivity and specificity of anti TTG IgA range from 38%-92.5% and 74%-97.5% respectively.[13-15] The reason why IgA is used in the diagnosis of CD is that, celiac disease prevalence is on the rise and there is a need of a diagnostic method in order to timely screen and manage these patients.[16]

The purpose of this study is to detect CD patients in our population with simple screening test because the prevalence of the disease is much higher as previously thought.[17] Serology is a useful adjunct to diagnosis and diagnostic criteria need to be developed appropriately for celiac disease in developing countries despite limited facilities.[8] Locally no studies are available. Such study has been done in Iran using histopathology as gold standard to determine accuracy of TTG IgA in celiac disease patients. If our study results show good accuracy then this test would be recommended for diagnosis of celiac disease.[18]

The objective of the study was to determine the accuracy of anti-tissue TTG IgA as a diagnostic tool to detect celiac disease in patient presenting with clinical features suggestive of the disease while keeping histopathology as a gold standard.

MATERIAL AND METHODS

This cross sectional study was carried out in Lahore at The Children's hospital Lahore. Sample size of 200 patients was calculated with 95% confidence level and 5% margin of error taking 16.6% expected prevalence of celiac disease in children.[8] Celiac disease is suspected in children with any of these at the time of presentation: 1) diarrhea (3 or more liquid stool/day) At least for 2 weeks; 2) hemoglobin <7gm/dl**;** 3) FTT is defined as weight for age that falls below the 5th percentile and; 4) height less then 5th centile. Boys and girls of suspected celiac disease (as per operational definition) between the ages of 6 months to 15 years and child who has been previously diagnosed as a case of celiac disease on the basis of biopsy or other antibodies like anti-gliadin, anti-reticulin and anti-endomysial antibodies were included in the research.

All patient with history of congenital heart disease, chronic lung (such as TB), liver disease (bilirubin >1.2), kidney diseases (creatinine >1.2), skeletal or CNS disease as a cause of growth retardation were excluded. Written consent was taken from guardians/parents. Cases were collected from the outdoor and indoor patient's department, having history and clinical examination suggestive of celiac disease. Then TTG IgA antibodies were done on 3 ml blood sample of patient by ELISA method. ISA and cut off value is >10u/ml is considered positive for CD. Report was finalized by pathologist. Data was recorded on proforma by researcher and then

small bowel biopsy was performed. Biopsy was performed at Children's Hospital Lahore by gastroenterologist. The report was verified by histopathologist of the same hospital.

True Positive: Patients in which TTG>10u/ml and histopathology positive for celiac disease:

True Negative: Patients in which TTG<10 u/ml and histopathology negative for celiac disease

False Positive: Patients in which TTG>10 u/ml and histopathology negative for celiac disease

False Negative: Patients in which TTG<10 u/ml and histopathology positive for celiac disease

Histopathology: Histopathology findings of any of these: villous atrophy or flat mucosa, crypt hyperplasia and intraepithelial lymphocytes were labeled as positive for CD.

Patients were labeled as positive or negative for celiac disease based on transglutaminase antibodies and histopathology. All data was entered on predesigned proforma.

Data was analyzed by using SPSS version-10. Relevant descriptive statistics were used. Frequency and percentage were computed to present categorical variables including gender, celiac disease presence and absence of CD on both modalities, family history of CD. Quantitative variables like age, weight and height were presented by mean ± SD. Statistical analysis was preformed to compute sensitivity, specificity, positive and negative predictive values by making 2x2 table taking histopathology as gold standard. Data was stratified for age and gender. Post-stratification chi square test was applied. P ≤0.05 was taken as significant.

RESULTS

A total of 200 cases fulfilling the inclusion/exclusion criteria were enrolled to find the accuracy of anti-tissue transglutaminase antibodies as a diagnostic tool in detection of CD. Age distribution of the patients was done which shows that 90% (n=180) were between 6m-10 years of age while only 10% (n=20) were between 11-15 years, mean ±sd was calculated as 6.74 ± 3.25 years (table 1). Patients were distributed according to gender showing 59% (n=118) were male and 41% (n=82) were female. Mean height was calculated as 113.28 ± 14.55 cm while weight was calculated as 18.45 ± 4.30 kg (table 1).

TABLE 1: Demographic data of children with celiac disease (n=200)

	No. of patients (%)	Mean ± SD
Age (in years)		
6m-10	180 (90.0)	6.74 ± 3
11-15	20 (10.0)	
Gender		
Male	118 (59.0)	-
Female	82 (41.0)	
Height (cm)	--	113.28 ± 14.55
Weight (kg)	--	18.45 ± 4.30

Family history of celiac disease was recorded in 6% (n=12) while 94% (n=188) had no family history. Frequency of celiac disease was calculated in 20.5% (n=41) while remaining 79.5% (n=159) had no findings of celiac disease (table 2). Diagnostic specificity of anti-tissue TTG IgA in detection of CD by keeping histopathology as gold standard was calculated showing sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate as 70.73%, 69.81%, 37.66%,90.24% and 70% for respectively (table 3).

TABLE 2: Frequency of celiac disease on histopathology (n=200)

Celiac Disease	No. of patients	Percentage
Yes	41	20.5
No	159	79.5
Total	**200**	**100.0**

TABLE 3: Diagnostic accuracy of anti-tissue TTG IgA in detection of celiac disease in patients suspected for celiac disease by keeping histopathology as gold standard (n=200)

TTG IgA	Histopathology	
Positive	True positive (a) 29 (14.5%)	False positive (b) 48 (24%)
Negative	False negative (c) 12 (6%)	True negative (d) 111 (55.5%)
Total	**a + c 41 (52.5%)**	**b + d 159 (79.5%)**

Sensitivity = a / (a + c) x 100 =70.73%
Specificity = d / (d + b) x 100 = 69.81%
Positive predictive value = a / (a + b) x 100 = 37.66%
Negative predictive value = d / (d + c) x 100 = 90.24%
Accuracy rate = a + d / (a + d + b + c) x 100 = 70%

DISCUSSION

Celiac disease is an autoimmune chronic gastrointestinal disorder in which ingestion of gliadin portion of gluten present in wheat, rye and barley causes the damage to mucosa of the small intestine in genetically susceptible individuals. The disease is associated with human leukocyte antigen (HLA)-DQ2/DQ8 in majority of cases, but only about 4% of them develop the disease after introduction of gluten diets. The diagnostic facilities have improved, the criteria have been revised with sophisticated serological investigations including TTG IgA antibodies followed by gold standard proximal intestinal biopsy.

We planned to detect CD patients in our population with simple screening test because the prevalence of the disease is much higher as previously thought.

In our study, out of 200 cases, 90% (n=180) were between 6m-10 years of age while only l10% (n=20) were between 11-15 years, mean $\pm$sd was calculated as 6.74$\pm$3.25 years, 59% (n=118) were male and 41% (n=82) were females, frequency of celiac disease was calculated in 20.5% (n=41) while remaining 79.5% (n=159). TTG IgA specificity in detection of CD by keeping histopathology as gold standard was calculated showing sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate as 70.73%, 69.81%, 37.66%, 90.24% and 70% for respectively.

Munir Akmal concluded that TTG IgA >25 U/ml was found to be 91.8% sensitive, 29.63% specific and 73.68% accurate in diagnosing celiac disease taking histopathology as gold standard with positive and negative predictive values of 76.54% and 57.4% respectively.[19] Fernandez investigated the diagnostic accuracy of six human tissue transglutaminase (based ELISA tests in Saharawi CD patients and recorded the sensitivity and specificity of TTG IgA Enzyme-Linked Immunosorbent Assay (ELISA) is 90% and 95% respectively,[13] which is higher than our study.

On the other hand, some other studies recorded the overall sensitivity and specificity of TTG IgA range from 38-92.5% and 74-97.5% respectively.[14-16] Our findings are in agreement with these findings and within an acceptable range.

Rabbani and colleagues[20] determined the accuracy of TTG IgA in the diagnosis of celiac disease, taking small bowel biopsy as a gold standard and calculated sensitivity, specificity, positive predictive value, negative predictive and accuracy, values 92.96%, 52.17%, 75%, 82.76% and 76.92% in study group, these findings are nearly agreed with our study.

Hashemi et al[21] found sensitivity, specificity, positive predictive value and diagnostic accuracy of TTG IgA as 88.6%, 94.2%, 88.6% and 94.2% respectively in a study conducted in Iran. Rawal et al[22] also reported a high sensitivity of TTG IgA (94.7%) for celiac disease.

The reason why IgA is used in the diagnosis of CD is that, celiac disease prevalence is on the rise and there is a need of a diagnostic method in order to timely screen and manage these patients. [17]

In light of the results of our study we are of the view that accuracy of TTG IgA in celiac disease patients is good and this simple test may be recommended for diagnosis of celiac disease to reduce the burden on the hospital and to avoid surgical biopsies.

Our findings are primary in our institute, further trials are required to validate our findings.

CONCLUSION

We concluded that anti tissue transglutaminase antibodies diagnostic accuracy in detection of CD in patients with features suggestive of CD is in an acceptable range and this tool may be adopted for primary investigation of the morbidity. However, further trials are required to validate our findings.

Conflict of interest: Nil

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