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ORIGINAL ARTICLE

Accuracy of Anti-Tissue Transglutaminase IgA Antibody in the Diagnosis of Paediatric Celiac Disease

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Abstract

Objective: To determine the accuracy of anti-tissue transglutaminase IgA (TTG) antibody titer in the diagnosis of celiac disease, taking small intestine histopathology as the gold standard.
Study Design: Cross-sectional analytical study.
Place and Duration of Study: Department of Paediatrics, Benazir Bhutto Hospital, Rawalpindi, from February to July 2013.
Methodology: Sixty patients aged 2 - 13 years, admitted in the Paediatric Department of Benazir Bhutto Hospital, Rawalpindi, having at least 3 features from chronic diarrhea, malnutrition, short stature, anemia, abdominal distension and clubbing, were included. Age, gender, weight and height were recorded. Abdominal distension and clubbing were clinically noted. For hemoglobin, blood complete picture was done. For determination of nutritional status and short stature, standard centile charts were used. TTG titer upper GI endoscopy, duodenal biopsy, and histopathology were done in all cases.
Results: There were 60 patients; 32 males, 28 females with mean age of 5.85 ±3.36 years. Frequency of CD was 63.33% in study population. Sensitivity of TTG was 86.84%, with 81.82% specificity, 89.19% positive predictive value, and 78.26% negative predictive value for diagnosing CD. TTG titre more than 50 iu/ml had a 100% positive predictive value.
Conclusion: TTG is an excellent screening test for the diagnosis of paediatric CD. TTG value > 50 IU/ml has 100% positive predictive value.

Key Words: *Celiac disease. Paediatrics. Histopathology.*

INTRODUCTION

Celiac disease (CD) is an immune-mediated disorder with genetic predisposition, characterized by chronic inflammation of the small intestine triggered by the ingestion of gluten containing grains; such as wheat, barley and rye. The overall prevalence of celiac disease is 1%.[1] In 2010, 2.2 million children under 5 years of age, were suffering from CD.[2]

Clinical manifestations of celiac disease are variable, ranging from asymptomatic to a diverse spectrum of fatigue, failure to thrive, diarrhea, anemia, clubbing, bloating and abdominal pain.[3] The hallmark of CD is intestinal damage, characterized by intraepithelial lymphocytes, villous atrophy, and crypt hyperplasia.[4]

Less common manifestations of celiac disease include dermatitis herpetiformis, a blistering rash; gluten ataxia and celiac crisis; a rare life-threatening disorder characterized by explosive diarrhea, hypoproteinemia, metabolic, and electrolyte imbalances.[5] Clinical presentation of CD can broadly be classified as gastrointestinal (classical, typical) and non-gastrointestinal (atypical); with non-gastrointestinal cases being increasingly recognized.[6]

Serological testing; antigliadin IgG antibody has diagnostic accuracy reaching 100% for the diagnosis of celiac disease in children less than 2 years of age.[7] TTG and anti-endomysial IgA (EMA) antibody testing have been shown to be highly sensitive and specific for celiac disease.[8] TTG and/or EMA have a high accuracy (sensitivity 90 - 98% and specificity 95 - 99%).[9] Histological studies are considered as the gold standard for establishing the diagnosis.[1,5,6,9,10]

Histological changes in small intestinal mucosa in celiac disease include intraepithelial lymphocytosis and villous atrophy.[11] Villous atrophy may be of variable degree ranging from mild to total villous atrophy. Abnormal morphologic features, such as cuboidalization, are seen on surface enterocytes less commonly, cytoplasmic vacuolization. Special stains show subepithelial thickening, consistent with minimal collagen deposition.[12]

Diagnosis of celiac disease is a matter of discrepancy. Some studies show very high sensitivity and specificity of anti-tissue transglutaminase (TTG), recommending it as sufficient evidence of starting gluten-free diet.[13] Others suggest strongly positive TTG (> 10 folds cut off) as diagnostic, and values less than 10 times need further confirmation by duodenal biopsy and histopatholgy.[14] Some studies have suggested using double

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anti-body testing with TTG and EMA.[16] Yet others have suggested using TTG anti-body and anti-gliadin anti-bodies together.[17]

The only satisfactory treatment for celiac disease is life-long strict adherence to a gluten-free diet. Prospective research is aimed at developing alternative therapies which may permit an unrestricted diet including proteolytic enzymes that degrade gluten, desensitizing vaccines, anti inflammatory drugs, polymeric binders, inhibitors of transglutaminase 2, and HLA-DQ2 blockers. However, these have not been proven effective enough to replace gluten-free diet.[18]

Early diagnosis of celiac disease is highly imperative to institute early intervention in order to prevent profound macronutrient and micronutrient deficiencies, as well as long-term complications. This study is planned to determine the accuracy of TTG in the diagnosis of celiac disease taking small intestinal histopathology as the gold standard, so as to avoid endoscopy and duodenal biopsy.

The objective of this study was to determine the diagnostic accuracy of anti-tissue trasglutaminase IgA antibody titer in diagnosis of CD, taking small intestinal histopathology as the gold standard.

METHODOLOGY

The cross-sectional validation study conducted on patients admitted in Department of Paediatrics, Benazir Bhutto Hospital, Rawalpindi. Duration of study from February till July 2013.

Sample size was 60 patients taking prevalence of disease in study population 61%,[19]. A 96.1% TTG sensitivity, specificity 99.8%,[14] desired precision 8% and confidence interval of 95%. Consecutive non-probability sampling method was used for sample selection.

Suspected cases of celiac disease; i.e. patients having 3 or more clinical features of celiac disease (chronic diarrhea, malnutrition (any degree), short stature, anemia, abdominal distension and clubbing) at time of admission, in operational definitions, aged 2 - 13 years, on gluten containing diet, were inducted. Patients already diagnosed with celiac disease or any other condition resulting in chronic diarrhea (e.g. cystic fibrosis, abdominal TB, chronic giardiasis) and those with chronic debilitating illness (cerebral palsy, neuro-muscular diseases) were excluded. After approval from hospital ethical committee, the purpose and procedure of the study was explained and consent was taken from parents/guardians.

Children aged between 2 and 13 years, admitted in Paediatric Department of Benazir Bhutto Hospital satisfying inclusion and exclusion criteria, were included in the study until the sample size was achieved. Data included age, gender, weight, percent weight of desired and blood complete picture. Clubbing and abdominal distension were clinically confirmed. Three ml blood was drawn from each patient by venous puncture; coagulated blood sample was subjected to analysis for TTG antibody titer from the same reference laboratory. Upper limit for TTG was taken as 7 IU/ml. All reports were verified by the same consultant pathologist. Upper GI endoscopy and duodenal biopsy of all patients were performed by a consultant gastroenterologist at Endoscopy Department, Benazir Bhutto Hospital. Histopathology of intestinal biopsy specimen was done by the same consultant histopathologist at Benazir Bhutto Hospital, Rawalpindi.

Computer program SPSS version 20 was used for data analysis. Descriptive statistics were calculated for all the variables. Qualitative variables including gender, true positive cases, presence of malnutrition, short stature, clubbing, and abdominal distension were presented as frequency and percentage. Quantitative variables including age, weight, and hemoglobin were presented as mean and SD. Tables and charts were made for qualitative variables.

A 2x2 contingency table was constructed to determine accuracy of anti-tissue transglutaminase as diagnostic test in terms of sensitivity and specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy. Diagnostic accuracy of more than 90% was considered excellent, and 80 - 90% as very good.

RESULTS

The total number of patients was 60 with age ranging from 2 to 13 years (mean=5.85 ±3.36 years), 32 (53.33%) males, and 28 (46.67%) females. All patients were malnourished.

A total number of 38 out of 60 patients were diagnosed with CD (true positive + false negative); giving a CD frequency of 63.33% in the study population. Mean age at diagnosis was 6.89 ±2.97 years, with 17 males and 21 females, giving a male to female ratio of 1:1.23. Only 12 (31.57%) patients diagnosed with CD presented with chronic diarrhea; while 26 (68.42%) patients presented without diarrhea. Results of the 2x2 table are described in Table III. Sensitivity of TTG was 86.84%, with specificity 81.82%, positive predictive value 89.19%, and negative predictive value 78.26%; for diagnosing CD. Diagnostic accuracy of TTG was 85%.

Among all TTG positive patients, 25 patients had TTG value more than 50 IU/ml. Each of these patients was a true positive with histopathological changes corresponding to Marsh 3b or higher grade lesion with 100% positive predictive value.

DISCUSSION

CD is a very common condition with a prevalence of biopsy proven disease as 1% worldwide.[1] The number of subclinical cases of CD is very high due to subtle

manifestations closely mimicking other common conditions, such as primary malnutrition and inflammatory bowel syndrome. Frequency of CD in study population of present study, which includes high-risk population (see inclusion criteria), was 63.33%. This result closely correlates with the results of Aziz *et al*. who studied similar high risk population in Karachi.[19]

Male to female ratio in the present study was 1:1.23 which is in close concordance with previous studies which also show a female preponderance.[20]

In present study, 68.42% patients diagnosed with CD had no chronic diarrhea at the time of presentation, with non-GI symptoms as the presenting features. Only 32.38% patients with CD presented with chronic diarrhea. This is a well recognized trend towards non-GI presentation observed worldwide, and results partly correlate with previous studies such as conducted by Ehsani-Ardakani *et al*.[20] Close association with chronic diarrhea, anemia and malnutrition is also well recognized.[27,28]

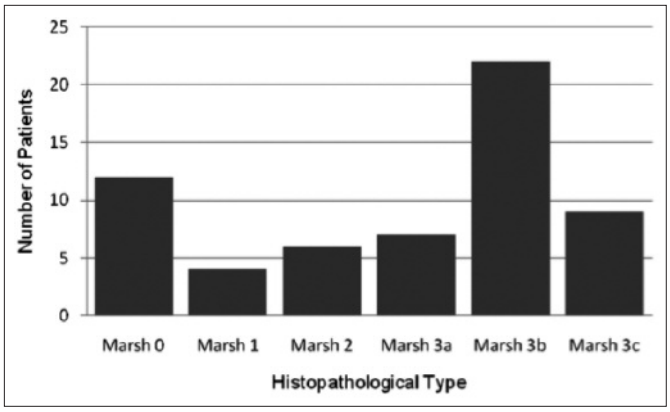

Figure 1: Histopathological distribution (Modified Marsh).

Table I: Clinical features of study population.

Clinical feature	Number of patients Total (n) = 60	Frequency (%)
Chronic diarrhea	20	33.33
Short stature	59	98.33
Anemia	42	70
Abdominal distension	28	46.67
Clubbing	15	25
Malnutrition	60	100
1st Degree	14	23.33
2nd Degree	30	50
3rd Degree	16	26.67

Table II: Distribution of patients in different groups.

Total	No.	Mean age ±SD	Male	Female
	60	5.85 ±3.36	32 (53.33%)	28 (46.67%)
True positive (a)	33	7.14 ±2.92	15 (45.04%)	18 (54.96%)
False positive (b)	4	2.38 ±0.48	3 (75%)	1 (25%)
False negative (c)	5	4.90 ±2.56	2 (40%)	3 (60%)
True negative (d)	18	4.68 ±3.71	12 (66.67%)	6 (33.33%)

Table III: 2x2 contingency table.

	Disease +ive (Histopathology)	Disease -ive (Histopathology)
TTG+ive	True positive a = 33	False positive b = 4
TTG-ive	False negative c = 5	True negative d = 18

In the present study, sensitivity of TTG was calculated to be 86.84%. Specificity of TTG was 81.82%. Positive predictive value was 89.19% and negative predictive value was 78.26%. These results appear representative as they closely correlate with the results of Samasca *et al*., showing a high sensitivity, specificity and positive predictive value,[8] but a relatively low negative predictive value. On the other hand, Alessio *et al*. and Bürgin-Wolff *et al*. demonstrated better sensitivity, specificity, positive and negative predictive values.[14,21]

In the present study, TTG value 7 times or more above the cut off limit (7 IU/ml) i.e. TTG value > 50 IU/ml was associated with villous atrophy and histological lesion of Marsh 3b or high grade with a positive predictive value of 100%. This finding can be compared with the results of Mubarak *et al*., who found a 100% positive predictive value with TTG > 100 IU/ml.[7] However, current study has shown this high positive predictive value at a much lower TTG (> 50 IU/ml) level than the result of previous study.

It is evident from the above discussion that TTG provides an excellent screening tool for CD with a very high sensitivity, specificity, positive predictive value and negative predictive value. Strongly positive TTG value, i.e. TTG > 50 IU/ml, provides a positive predictive value of 100%; suggesting sufficient diagnostic accuracy to obviate the need for endoscopic duodenal biopsy and histopathologic examination of duodenal mucosa. Therefore, a strongly positive TTG is diagnostic of CD.

Histopathology is the time tested gold standard for the diagnosis of CD. However, it requires necessary expertise and costly equipment, which is not freely available in many centers of the country. Endoscopy and duodenal mucosal histopathology should be reserved for cases with TTG positivity < 50 IU/ml, and TTG negative cases with strong clinical suspicion of CD. In doubtful cases, a biopsy check should be performed after 6 weeks of GFD to demonstrate mucosal healing after gluten withdrawal.

It is evident from the discussion that diagnosis is the most key step in the management of CD. This is because of protean clinical features of CD, which are at times very diverse and subtle. Scenario is made more complex by large number of sub-clinical cases having no clinical features. Early diagnosis is of utmost importance in determining the course of disease and possible complications. Being a potentially curable condition, makes early diagnosis even more important.

Therefore, it is wise to screen all patients who have any combination of clinical features which may be attributable to CD for possible CD. In present study, patients having minimum of 3 clinical features out of 6 (chronic diarrhea, malnutrition, short stature, anemia, abdominal distension, clubbing) were included, and resultant frequency of study population was 63.33%. Therefore, it can be safely recommended that patients having even fewer clinical features should be screened by TTG to detect CD patients with minimal signs and symptoms.

Worldwide, focus is shifting towards diagnosis of sub-clinical cases by screening high risk population. Serological testing plays a key role in screening of at risk population. High risk population such as relatives of CD patients, patients with Down syndrome, Turner syndrome, type 1 diabetes, thyroid dysfunction, cryptic hypertransaminasemia should be screened for CD by TTG testing irrespective of presence or absence of clinical features of CD.

CONCLUSION

TTG is an excellent screening test for CD in high risk paediatric population having 81.08% sensitivity, 86.96% specificity, 90.91% positive predictive value, 74.07% negative predictive value and a diagnostic accuracy of 85%. TTG value more than 50 IU/ml has a 100% positive predictive value for Marsh 3b or higher lesion on small intestinal biopsy.

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