DIAGNOSTIC ACCURACY OF IGA ANTI-TISSUE TRANSGLUTAMINASE ANTIBODY IN THE DIAGNOSIS OF CELIAC DISEASE TAKING HISTOPATHOLOGY AS GOLD STANDARD

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ABSTRACT

Objective: To determine the diagnostic accuracy of IgA anti-tissue transglutaminase antibody in the diagnosis of celiac disease taking histopathology as gold standard.

Study Design: Cross-sectional validation study.

Place and Duration of Study: Department of Pediatrics, Pak Emirates Military Hospital Rawalpindi, from Apr 2015 to Jul 2016.

Methodology: Ninety five consecutive children presenting with suspicion of celiac disease were included into this study after taking written informed consent. A predesigned proforma was used to record patient's demographic details. Anti-tTG level of ≥25 U/ml was taken as diagnostic of celiac disease while results of histopathology on endoscopic biopsy were taken as gold standard.

Results: The mean age of the patients was 6.48 ± 3.20 years and majority 53 (55.8%) of the children were aged between 5-10 years. There were 47 (49.5%) male and 48 (50.5%) female children in the study group. The serum Anti-tTG level ranged from 8.0 U/ml to 759.0 U/ml with a mean of 298.75 ± 225.51 U/ml. Taking a cut-off value of ≥25 U/ml for anti-tTG, 81 (85.3%) children were suspected of celiac disease. Histopathology of endoscopic biopsy confirmed celiac disease in 68 (71.6%) children with 62 true positive, 19 false positive, 6 false negative and 8 true negative cases. It yielded 91.18% sensitivity, 29.63% specificity and 73.68% accuracy for anti-tTG (≥25 U/ml) in the diagnosis of celiac disease with positive and negative predictive values of 76.54% and 57.14% respectively.

Conclusion: IgA anti-tissue transglutaminase antibody (≥25 U/ml) was found to be 91.18% sensitive, 29.63% specific and 73.68% accurate in the diagnosis of celiac disease taking histopathology as gold standard with positive and negative predictive values of 76.54% and 57.14% respectively.

Keywords: Anti-Tissue Transglutaminase antibody, Celiac disease, Diagnostic accuracy.

INTRODUCTION

Endoscopic small bowel biopsy is the current 'gold standard' for the diagnosis of celiac disease (CD) however, the severity of injury ranges from Marsh I to Marsh IV and only Marsh stages III and IV have largely been considered to be indicative of celiac disease [1,2]. But, endoscopy and duodenal biopsy is cumbersome, painful and costly[2]. The dawn of serological tests, therefore, offered the ever welcomed opportunity of diagnosing celiac disease without the need for endoscopy. The detection of auto-antibodies is frequently used as a first-line test to recognize individuals who might need a duodenal biopsy[1,2]. Historically, screening for celiac disease has been performed with a diagnostic test for malabsorption (D-xylose) or serological tests for anti-endomysial antibodies (anti-EMA) and anti-gliadin antibodies (AGA). These serological tests have become vital in the identification and diagnosis of celiac disease[2]. After Dieterich *et al* revealed that tTG was the main (or the sole) auto antigen recognized by anti-endomysial antibody (EmAs) in CD patients, the use of an ELISA based on tTG was suggested and widely established for the diagnostic assessment of such patients[3]. Anti-tTG testing is highly sensitive and remains the single serological test of choice for diagnosis and screening of celiac disease[4]. It is notable that tTG antibody levels fluctuate depending on the degree of intestinal damage. Thus, a negative test is likely in a patient with marginal pathology (i.e, Marsh I lesion) and does not essentially rule out milder forms of celiac disease. Repeat testing may offer important clinical insight and help decide the appropriate timing for a biopsy or observing dietary compliance[2]. The reported specificity of anti-tTG greatly varies in the existing literature from as low as 9.5%[5] to as high as 100%[6]. With a low specificity of 9.5% anti-tTG may not be a good diagnostic tool and should only be used for screening while with a high specificity of 100% it can confirm the celiac disease without the need for endoscopic biopsy.

Due to conflicting international and limited local published material, the purpose of the current study was to evaluate the role of anti-tTG antibody in the diagnosis of celiac disease with a hope that the results

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of the present study may provide a screening or diagnostic tool limiting the need for endoscopic biopsy in children suspected of celiac disease.

METHODOLOGY

This was a cross sectional validation study conducted at the department of Pediatrics, Combined Military Hospital Rawalpindi, from April 2015 to July 2016. Non-probability, consecutive sampling was used. Ninety five consecutive children of both genders aged between 2-14 years presenting with persistent symptoms, either one or any combination of persistent diarrhea, abdominal pain, vomiting, abdominal distension, constipation, failure to thrive, idiopathic short stature, anaemia and lassitude/weakness were included into this study after taking written informed consent from parents or legal guardian of the patient. Patients already diagnosed with Celiac Disease or other disorders resulting in chronic malabsorption; such as cystic fibrosis, immunodeficiency and chronic giardiasis; patients with neuromuscular disorders, cerebral palsy; and those who did not give consent were excluded from the study.

Serum anti-tTG levels were acquired and a cut off value of ≥25 U/ml was taken as diagnostic of celiac disease (upper limit of reference value from our laboratory)[7]. These patients underwent endoscopic biopsy of the duodenal mucosa. Marsh-Oberhuber grading was used. Results of histopathology (Marsh III) were taken as gold standard and the diagnostic accuracy of anti-tTG was judged accordingly. Children with inflammatory bowel disease, abdominal tuberculosis, cystic fibrosis, and other established causes of FTT and short stature were excluded from the study. Exclusion criteria written in two separate paras; please merge them. Also must exclude children already on wheat free diet as these children have lower tTG levels. A predesigned proforma was used to record patient's demographic details, anti-tTG and histological diagno-sis.

All the patients were assessed by a single consultant pediatrician and all the histological reporting were acquired from the same lab to eliminate bias. Numerical variables i.e. age, serum Anti-tTG level have been presented by Mean ± SD while frequency and percentage has been calculated for categorical variables i.e. gender and histopathological findings. A 2x2 contingency table has been generated to calculate sensitivity, specificity, accuracy and positive and negative predictive values of serum Anti-tTG level in the diagnosis of celiac disease taking histopathology as gold standard.

RESULTS

The age of the patients ranged from 7 month to 14 years with a mean of 6.48 ± 3.20 years. Majority 53 (55.8%) of the children were aged between 5-10 years followed by 34 (35.8%) children aged under 5 years and 8 (8.4%) children aged between 10-14 years. There were 47 (49.5%) male and 48 (50.5%) female children. The serum Anti-tTG level ranged from 8.0 U/ml to 759.0 U/ml with a mean of 298.75 ± 225.51 U/ml. By taking a cut-off value of ≥25 U/ml for anti-tTG, 81 (85.3%) children were suspected of celiac disease.

Results of histopathology have been shown in table-I. Histopathology of endometrial biopsy confirmed celiac disease in 68 (71.6%) children with 62 true positive, 19 false positive, 6 false negative and 8 true negative cases. It yielded 91.18% sensitivity, 29.63% specificity and 73.68% accuracy for anti-tTG (≥25 U/ml) in the diagnosis of celiac disease with positive and negative predictive values of 76.54% and 57.14% respectively (table-II). An ROC curve was plotted which revealed an area under curve to be 0.772 (p=0.000).

Table-I: Histological findings on duodenal biopsy.

Histological Findings	n (%)
Unremarkable Duodenal Mucosa	4 (4.2%)
Focal villous atrophy Marsh III a	7 (7.4%)
Partial villous atrophy Marsh III b	36 (37.9%)
Complete villous atrophy Marsh III c	25 (26.3%)
Mild Non-specific Duodenitis	21 (22.1%)
Giardiasis	2 (2.1%)

Table-II: Diagnostic accuracy of anti-tTG.

Anti-tTG Diagnosis	Histopathological Diagnosis	
	Celiac Disease	No
Celiac Disease	62	19
No	6	8

Statistic	Value
Sensitivity	91.18%
Specificity	29.63%
Accuracy	73.68%
Positive Predictive Value	76.54%
Negative Predictive Value	57.14%

DISCUSSION

The immune response in celiac disease involves the production of antibodies against the intestinal enzyme Tissue Transglutaminase. These auto antibodies are either immunoglobulin G (IgG) or immunoglobulin A (IgA). The level of anti tTG IgA in the blood is more reliable for the detection of disease because it is formed in the small intestine, where gluten is responsible for inflammation in gluten-sensitive people[8]. In the present study, the mean age of the patients was

6.48 ± 3.20 years with nearly equal gender distribution. Babar *et al*[9] in 2011 reported similar mean age of 6.35 ± 2.83 years in pediatric patients presenting at Sheikh Zayed Hospital, Rahim Yar Khan. Jamro *et al*[10] (2012), Rabia *et al*[11] (2012) and Ikram *et al*[12] (2011) reported

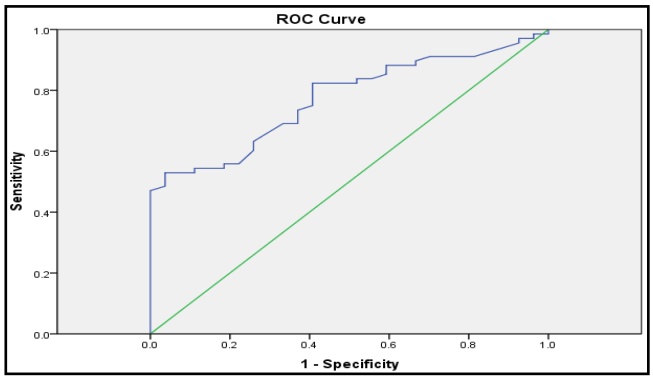

Figure-1: ROC Curve.

relatively higher mean age of 7.11 ± 2.8 years, 8 ± 3 years and 8.9 ± 3.7 years respectively. Much higher mean age of 11 ± 3.6 years has been reported previously by Makharia *et al*[13] in Indian such children. Rabbani *et al*[8] (2011) reported similar gender (m:f) distribution (52% vs. 48%) in children presenting at The Children Hospital & The institute of Child Health, Multan, Pakistan. Emami *et al* 14 (62%) and Dutta *et al* 15 (59.8%) reported slight male predominance among Irani and Indian such patients while Jamro *et al*[10] reported a female predominance (71.4%) in Pakistani such children. In the present study, the prevalence of celiac disease was 71.58% in study population; i.e. patients with clinical features of Celiac Disease. Hashmi *et al*[16] (2016) reported similar disease prevalence of 63.33% in children presenting at Benazir Bhutto Hospital, Rawalpindi. Javaeed *et al*[17] (2016) however reported much lower prevalence of 11.57% among adults presenting at Khyber Medical University, Peshawar. Eremic *et al*[6] (2012) and Emami *et al*[14]. (2008) reported similar prevalence of 8% and 6% in Serbian and Irani such children.

Partial villous atrophy (Marsh IIIb) was the most frequent histological diagnosis (37.9%) followed by complete (Marsh IIIc, 26.3%) and focal villous atrophy (Marsh IIIa, 7.4%). Normal duodenal mucosa was reported in 4 (4.2%) children while 21 (22.1%) children had mild non-specific duodenitis. Dutta *et al*[15] (2008) reported Marsh IIIc being the most frequent finding (38.39%) followed by Marsh IIIb (33.34%) and normal mucosa (22.22%) in Indian patients of celiac disease. Emami *et al*[14] (2008) reported Marsh IIIa and Marsh IIIb being the most frequent histological findings in 38% of Irani such patients followed by Marsh IIIc (24%). Jamro *et al*[10] reported March II (64.3%) and Marsh III (35.7%) being the most common histological diagnosis in such patients at Shaheed Mohtrama Benazir Bhutto Hospital, Sukkar, Pakistan. IgA anti-tissue transglutami-nase antibody (≥25 U/ml) was found to be 91.18% sensitive, 29.63% specific and 73.68% accurate in the diagnosis of celiac disease taking histopathology as gold standard with positive and negative predictive values of 76.54% and 57.14% respectively. The results of the present study are similar to those of Bayram *et al*[5] (2015) who reported similar higher sensitivity (93.3%) but low specificity (9.5%) of anti-tTG in Turkish children. Better sensitivity and specificity has been reported in other local studies where Hashmi *et al*[16] reported 86.84% sensitivity and 81.82% specificity and Javaeed *et al*[17] reported 85.70% sensitivity and 99.1% specificity of anti-tTG in Pakistani children with celiac disease. Dutta *et al*[15] also reported better sensitivity and specificity of 77.8% and 89.1% respectively in Indian population. Eremic *et al*[6] reported much higher specificity of 100% with relatively lower sensitivity of 75% in Serbian population.

The low specificity observed in the present study can be partly due to the difference of laboratory test systems. Another explanation for this difference from previous studies can be the hypothesis that proteins other than tissue transglutaminase (tTG) may act as antigens in some patients for anti-tTG antibodies[18]. Based on the results of the present study, high sensitivity and low specificity of anti-tTG make it a better screening tool where positive children should undergo endoscopic biopsy for the confirmation of diagnosis.

CONCLUSION

IgA anti-tissue transglutaminase antibody (≥25 U/ml) was found to be 91.18% sensitive, 29.63% specific and 73.68% accurate in the diagnosis of celiac disease taking histopathology as gold standard with positive and negative predictive values of 76.54% and 57.14% respectively.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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