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Diagnostic Efficacy of Anti-Tissue Transglutaminase IgA Levels Exceeding Five Times The Upper Limit of Normal In Pediatric Celiac Disease At A Tertiary Care Centre

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ABSTRACT

Objective: To determine the diagnostic efficacy of anti-tissue transglutaminase IgA (tTG-IgA) levels exceeding 5 times the upper limit of normal (ULN) in children with celiac disease referred to a tertiary care centre.

Study Design: Cross-sectional validation study.

Place and Duration of Study: Combined Military Hospital (CMH), Rawalpindi, Pakistan, from Feb 2022 to May 2023 for 15 months.

Methodology: Children ≤ 16 years presenting in Paediatric Outpatient Department and admitted to Paediatric ward of Combined Military Hospital, Rawalpindi, Pakistan, with clinical features of chronic diarrhoea, abdominal distension, failure to thrive, pain in abdomen, vomiting, constipation, and pallor were included in the study. Demographic details, age, gender, weight, and height were recorded. Abdominal distension and anaemia were recorded clinically. Blood samples were withdrawn for haemoglobin and anti-tTG IgA titre. Upper gastrointestinal endoscopy was performed, and duodenal biopsy samples were sent for histopathology to pathologist.

Results: Total number of patients was 60. There were 25 (41.6%) males and 35 (58.3%) females. The Median age was 4.7 (7.00 – 3.00) years. Out of 60 patients, 45(75%) had celiac disease. Sensitivity of anti-tTG IgA more than 5 times upper limit of normal was 77.7%, specificity was 60%, positive predictive value was 85.3% and negative predictive value was 47.3% for diagnosing Celiac disease.

Conclusion: Anti-tTG IgA values > 50IU/ml have an 85.3% positive predictive value for diagnosis of celiac disease. Hence such patients can be exempted from invasive endoscopic procedure and histological confirmation.

Keywords: Celiac disease, Duodenal Biopsy, Histopathology, Paediatrics, Serology

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INTRODUCTION

Celiac disease (CD) is a chronic autoimmune disorder triggered by the ingestion of gluten-containing grains in genetically susceptible individuals.[1] It affects approximately 1% of the global population, making it one of the most common food-related disorders worldwide.[2] Accurate and timely diagnosis of CD is crucial to ensure proper management and prevent long-term complications.[3] Currently, the diagnostic methods available for CD include serological tests, endoscopic biopsies, and histological analysis of small intestinal tissue samples.[4] The gold standard for diagnosis has traditionally been the combination of positive serology and characteristic intestinal histology.[1] However, recent research has highlighted the potential of alternative approaches that do not involve invasive procedures.

The no-biopsy pathway, also known as the "diagnose-and-confirm" strategy, has emerged as a promising alternative to the traditional diagnostic approach.[5] It proposes that a diagnosis of CD can be established solely based on positive serological markers, without the need for confirmatory biopsies. This approach offers significant advantages, including reducing the burden on patients, minimizing healthcare costs, and expediting the diagnostic process.[3] It was first proposed by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) in 2012 for children who fall under a certain criterion. Later, it was endorsed and expanded to include more children in 20191.In many countries, diagnosis still depends upon the intestinal biopsy.[6] Also, in adults no-biopsy pathway is not widely recognised yet, and biopsy is considered most essential.[7,8] Implementing the no-biopsy pathway requires a comprehensive understanding of its pros and cons. On one hand, avoiding biopsies eliminates the discomfort and potential complications associated with endoscopic procedures. It also saves time and reduces costs, making it a more acceptable option for

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patients.[5] On the other hand, relying solely on serological markers may lead to misdiagnosis in some cases, particularly in individuals with atypical or mild forms of celiac disease. Especially in patients with IgA deficiency, serology is inconclusive.[9] This potential drawback emphasizes the need for further research to validate the efficacy and reliability of the no-biopsy pathway.

The previous studies have been largely about the diagnostic efficacy of anti-tTG IgA with the cut-off of 10 times the upper limit of normal.[6,10] The primary objective of this study is to determine the diagnostic efficacy of anti-TTG IgA levels exceeding five times the upper limit of normal in children with celiac disease referred to a tertiary care hospital. By evaluating the performance of this serological marker in a large cohort, the study aims to provide robust evidence supporting its use as a reliable diagnostic tool. Furthermore, this study seeks to contribute to the reinforcement of the no-biopsy pathway, enabling healthcare providers to make informed decisions regarding celiac disease diagnosis.

METHODOLOGY

A cross-sectional validation study was performed in outpatient as well as admitted patients in Combined Military Hospital, Rawalpindi, Pakistan, from February 2022 to May 2023. Permission was taken from Ethical Committee/Institutional Review Board of the hospital to conduct the research. After the approval (IRB certificate number: 341), sample size was calculated using WHO calculator, keeping the prevalence of celiac disease of 1.4% .[11] The estimated sample size required for the study was 22. A consecutive non-probability sampling method was used for sample selection. The children meeting the predefined inclusion and exclusion criteria were included in the study.

Inclusion Criteria: Children aged 16 years and younger who presented with clinical features including chronic diarrhoea, weight and/or height less than the 3rd percentile, iron deficiency anaemia, protruding abdomen, and clubbing.

Exclusion Criteria: Patients diagnosed with other chronic conditions like inflammatory bowel disease, abdominal tuberculosis, giardiasis, etc., were excluded from the study. Patients whose IgA levels were deficient, also excluded from study.

The procedure of the study was explained to the parents/guardians of the patients to obtain consent for data collection procedure. Data was collected from suspected cases of celiac disease, including age, gender, weight, and clinical symptoms. Patients were examined for abdominal distension and clubbing. The anti-tissue transglutaminase IgA (anti-tTG IgA) levels along with serum IgA levels of the patients, were sent for serology. An appointment for endoscopy was given, and their histological tests were obtained. Histopathology of duodenal biopsy showing villous atrophy and intraepithelial lymphocytes > 30 (IEL >30) was taken as a diagnostic criterion for the diagnosis of celiac disease. Three ml of blood sample was collected from each patient, and coagulated blood was sent to the same reference laboratory. Upper GI endoscopy was done by a Paediatric gastroenterologist in CMH, and histopathology samples were sent again to the same reference laboratory.Histopathological confirmation was done by a Pathologist at CMH, Rawalpindi, Pakistan.

The data was analysed using descriptive statistics by calculating frequency, percentages for qualitative variables. For quantitative variables like age median was calculated. The association between different categories was measured using the Chi-square test, and a *p*-value <0.05 was considered statistically significant. Statistical analysis and data interpretation were carried out by using the Statistical Package for the Social Sciences (SPSS) version 20.0 by IBM, USA.

RESULTS

Sixty patients were included in the study, with ages ranging from one and a half to thirteen years. Median age was 4.7 years (7.00 – 3.00). There were 25(41.6%) males and 35(58.3%) females, giving a male-to-female ratio of 1:1.4. Out of 60 patients, 45 were diagnosed with celiac (true positive + false negative), giving celiac disease a frequency of 75%. The mean age at diagnosis was 5.5±3.1 years. Out of 60 patients, 41 had anti-tTG IgA levels more than 5 times ULN. Out of them, 35 patients (85.3%) had positive histopathology for celiac disease, while 6 patients (14.6%) had a negative histopathology report. 7 out of the total 60 patients had anti-tTG IgA levels less than 5 times ULN. Of them, 4 patients (57.1%) had a positive biopsy, while 3 patients (42%) had a negative biopsy report. The association between the anti-tTG levels more than 5 times ULN and positive histopathology for celiac was statistically significant (*p*-value<0.05). (Table-I) Sensitivity of anti-tTG IgA more than 5 times ULN was 77.7%, specificity was 60 %, Positive predictive value (PPV) was 85.3 %, and Negative

Predictive value (NPV) was 47.3 %. (Table-II). About 29(64.4%) of the celiac disease patients presented with pallor while 23(51%) had chronic diarrhea, 11(24.4%) had pain in abdomen, 14(23%) had abdominal distension, 11(24.4%) had short stature, 6(13.3.%) had vomiting (12.8%) and 4(8.8%) had constipation as their clinical presentation. (Table-III)

Table-I: Comparison of Anti-Ttg Iga with Histopathology (n=60)

	tTG IgA > 5 times ULN (n=41)	tTG IgA < 5 times ULN (n=19)	*p*-value
Biopsy positive n (%)	35 (85.3%)	10(52.6%)	0.006
Biopsy negative n (%)	6 (14.6)	9(47.4%)	

Table-II: Sensitivity, Specificity, Ppv, And Npv of Anti-Ttg Iga > 5 Times Uln

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Anti-tTG IgA > 5 times ULN	77.7	60	85.3	47.3

Table-III: Frequency of Clinical Features In Celiac Disease (n=45)

Clinical Features	Number of patients & Frequency (%)
Chronic diarrhea	23(51)
Protuberant abdomen	14(23)
Pain in abdomen	11(24.4)
Constipation	4(8.8)
Vomiting	6(13.3)
Pallor	29(64.4)
Short Stature	11(24.4)

DISCUSSION

The study confirms that Anti-TTG IgA titers greater than five times the upper limit of normal (ULN) had an 85.3% likelihood of resulting in a positive biopsy diagnosis for Celiac Disease. Thus, validating the ESPGHAN guidelines,[12] which recommend a non-biopsy pathway in patients with high anti-tTG IgA titers. The study also suggests that the limitations of endoscopic biopsy sampling may account for some of the negative results. If the no-biopsy approach is to be implemented for patients with anti-tTG IgA levels exceeding 5x ULN in our medical center, approximately 41 out of 60 patients would avoid the need for an endoscopy. This non-invasive diagnostic approach would not only enhance the cost-effectiveness of hospital services but would also improve the overall patient satisfaction by limiting invasive diagnostic tests.

These findings align with the results of the studies compared. The association of anti-tTG IgA value of more than 5 times upper limit of normal i.e. 50 IU/ml with a positive biopsy report for celiac disease (i.e. findings of villous atrophy and intraepithelial cells >30)in about 85.3 % of cases, although considered good, falls slightly short of the results from a study conducted by Siba Prosad Paul and colleagues. In this research, anti-tTG IgA levels exceeding 5 times ULN were linked to histological confirmation of celiac disease with a higher positive predictive value of 99.5%.[13] Smarrazzo et al., have also reported good diagnostic accuracy, with positive predictive values ranging from 95% to 96% for anti-TTG IgA levels exceeding 10 times ULN.[6] However, Johnston et al., have highlighted that individuals with low-titer anti-tTG IgA antibodies benefited from undergoing a biopsy assessment, as 42% of them did not exhibit histological features of Celiac disease. This result is higher than this study, in which 25% of patients with low anti-tTG IgA levels had negative biopsy findings.[10] Therefore, it is evident that endoscopy plays a crucial role in evaluating patients with low anti-tTG IgA titers.

Since this study's inclusion criteria were based on a high-risk population, that's why prevalence noted was high, i.e., 75%. Hashmi et al conducted a study in 2013 in which 38 out of 60 patients were diagnosed with celiac, giving a prevalence of 63.3 %.[14] The study showed a slightly higher prevalence than this research. The male-to-female ratio in the current study was 1:1.4, which is very similar to the study conducted by Gulcu *et al.*, showing female predominance.[15] Although the difference in the gender distribution in the current study remained unfocused and can be explored in future studies as Celiac is immune-mediated and occurs in genetically susceptible individuals. In this study, the mean age of diagnosis was 5.5 years (±3.1). This finding closely relates with the research conducted by Ashtari et al. and El-Metwally et al., both of which reported a slightly younger mean age of diagnosis, ranging from 1 to 3 years.[16,17]

The most common symptom at presentation in this study was pallor, which is also highlighted by Isa HM *et al.,* in which author establishes the fact that with an increase in prevalence of the disease, its clinical presentation changes. Children with celiac disease presented most with pallor, abdominal distension, and failure to thrive in the order of higher to lower frequency.[11] In this research study, pallor was either the sole manifestation or present with other features. Some children had severe anaemia and were transfused with blood. Anaemia in celiac disease is multifactorial and mainly due to iron malabsorption, while vitamin B12 and Folate deficiency are other prominent causes that can play a role. A study conducted by Bledsoe *et al.,* showed most common presentations to be abdominal pain, growth retardation, pallor and abdominal distension.[18] The second most common clinical presentation in this study was chronic diarrhoea. This is the 'classic' manifestation caused by interaction between inflammatory immune response and gluten peptides, including T-cell activation in the lamina propria of the intestine, as well as due to the role of B cells and microbiome. Similar insights have been given by Rostom *et al.,* who conducted the study on diagnostic accuracy through serological investigations.[19]

In the present study sensitivity of anti-tTG IgA, more than 5 times ULN, was calculated to be 77.7%. This result is near the results shown by Rostom *et al.,* in a study where sensitivity between 80-90 % was recorded in children.[19] In a study conducted by Meena *et al.,* in India, tTG IgA of 6.4 times ULN had 76% sensitivity.[20] The specificity of Anti-tTG IgA in our study was 60%. This higher sensitivity and lower specificity could be due to verification bias, as parents of many children with negative anti-tTG IgA refused to proceed towards endoscopy and hence could not be included in our calculation. The results of the current study endorse that although anti-tTG IgA has been an effective tool to screen for celiac disease in the past, it can also be used for the diagnosis of celiac disease in selective cases if the test result is more than 5 times the upper limit of normal. Taneja *et al* conducted a study showing a significant association between anti-tTG IgA titers and Marsh grading.[21] According to this study sensitivity and specificity of anti-tTG IgA, about 8.4 times ULN, were 91.7% and 68.4%, respectively, which compares well with our study.

Since endoscopy is an invasive procedure and not widely available in developing countries like Pakistan, it can be skipped in paediatric patients with anti-tTG IgA > 5 times ULN, and these children can be directly started on Gluten gluten-free diet. A huge chunk of celiac disease patients go undiagnosed, especially socioeconomically deprived children. It's a need of the hour to implement widespread application of anti-tTG IgA test for screening and diagnosis to minimise the underdiagnosis of celiac disease. This practice will also lower the burden on health care centres where the facility of endoscopy is available, but there is a huge load of patients on waiting lists for different diagnostic and therapeutic procedures. A major chunk of these patients, who are suspected celiac patients, can be exempted from endoscopy, and an early treatment can be started, based on anti-tTG IgA levels.

Meijer et al. have highlighted the use of a non-biopsy approach in the Netherlands, which has resulted in a reduction of biopsy rates by half.[22] The use of such non-invasive and readily available testing will increase the diagnostic yield and pave the way for early treatment with a gluten-free diet. This can help prevent major complications of celiac disease in patients and improve children's overall health through better growth and development.

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CONCLUSION

The study confirms the fact that patients with anti-tTG IgA levels exceeding 50 IU/mL demonstrate a high positive predictive value (85.3%) for celiac disease, supporting a non-invasive diagnostic approach. In such cases, the strong serological evidence may mitigate the need for invasive procedures like endoscopy, thus reducing patient psychological and financial burden without compromising diagnostic accuracy.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SI & FI: Data acquisition, data analysis, critical review, approval of the final version to be published.

AS & RN: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

B & HK: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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