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Frequency of TTG Positivity in Diarrhea Predominant IBS Patients at Peshawar, Pakistan

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ABSTRACT

Irritable Bowel Syndrome is abdominal discomfort and alterations in bowel habits without an identifiable organic cause. **Objective:** To determine the frequency of tissue transglutaminase positivity in diarrhea-predominant IBS patients. **Methods:** This cross-sectional study was done from 1st January 2021 to 30th July 2021 in the Medicine department of Khyber Teaching Hospital, Peshawar, Pakistan. Patients of either gender aged between 18-60 years presenting in the medical outpatient department with diarrhea-predominant irritable bowel syndrome. Diarrhea-predominant irritable bowel syndrome was defined as a patient who fulfilled the Rome-IV criteria for irritable bowel syndrome with ≥2 of the features. Tissue transglutaminase antibodies were done for all patients and tissue transglutaminase positivity was defined as anti-tissue transglutaminase IgA or IgG antibody levels ≥10 AU/ml on laboratory tests performed by the chemi-luminescence immunoassay technique. **Results:** In a total of 96 patients, 59(61.5%) were male. The mean age, weight, and duration of diarrhea were 37.64 ± 6.28 years, 81.48 ± 7.70 kg, and 11.67 ± 3.81 months, respectively. The tissue transglutaminase positivity was seen in 12(12.5%) of patients. Duration of disease above 6 weeks was found to have a significant association with tissue transglutaminase positivity ($p<0.001$) as all tissue transglutaminase-positive patients had a duration of disease above 6 months. **Conclusions:** It was concluded that the frequency of tissue transglutaminase positivity was high in diarrhea-predominant irritable bowel syndrome patients. Screening for celiac disease in irritable bowel syndrome patients can be worth considering especially in cases with relatively longer duration of irritable bowel syndrome diarrhea predominant symptoms.

INTRODUCTION

Irritable Bowel Syndrome (IBS) is described as abdominal discomfort and alterations in bowel habits without an identifiable organic cause, affecting approximately 10–25% of adults and adolescents [1, 2]. However, only 30% of individuals experiencing these symptoms seek medical consultation [3]. As the exact pathophysiology of IBS is not fully known, biopsychosocial factors, luminal factors like diet, and gut microbial flora are believed to play crucial roles [4]. IBS lacks clear diagnostic markers, making diagnosis based on clinical presentation as symptoms are not distinctive, leading to the proposal of clinical criteria for the diagnosis of IBS. Celiac Disease (CD), also referred to as "Celiac sprue", is an inflammatory condition affecting the small bowels, triggered by gluten found in cereals such as wheat, rye, barley, and oats, especially in individuals with genetic predisposition [5]. CD typically presents with classic symptoms including weight loss, malabsorption, chronic diarrhea, and abdominal pain [6]. The diagnosis of CD relies on laboratory and histopathological studies, even when the overt clinical symptoms are absent. "Immunoglobulin A (IgA) anti-tissue Transglutaminase (IgA-TTG)" tests have high sensitivity and specificity for the confirmation of CD. The diverse presentation of CD may significantly converge with IBS, but untreated CD does not improve with standard therapy for IBS and may pose an increased risk of severe complications [5]. A study from Egypt reported 14.2% of adult patients experiencing chronic non-bloody diarrhea to have CD. The diagnosis was confirmed through positive findings in IgA anti-TTG, histopathology, and a positive response to a gluten-free diet [7]. Some physicians advocate for routine CD screening for IBS, though it is not universally accepted. Diarrhea pre-dominant irritable bowel syndrome is a diagnosis of exclusion and celiac disease can be missed if

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patients are not screened for gluten sensitivity. We hypothesized that the frequency of TTG positivity is high in diarrhea-predominant IBS patients.

The study aimed to determine the frequency of TTG positivity in diarrhea-predominant IBS patients.

METHODS

This cross-sectional study was done at the Department of General Medicine, "Khyber Teaching Hospital, Peshawar", Pakistan, from January 2021 to July 2021. Approval from the "Institutional Research and Ethical Board" of Khyber Medical College, Peshawar, Pakistan was acquired (letter number: 392/DME/KMC). A sample size of 96 was calculated using the WHO sample size software, considering the expected frequency of TTG positivity at 14.2% in chronic diarrhea patients [7], with a 95% confidence level and 7% margin of error. A non-probability purposive sampling technique was adopted. Patients aged 18-60 years and presenting in the medical outpatient department with diarrhea-predominant IBS were included. The exclusion criteria were patients with a history of bloody diarrhea, diarrhea associated with fever, a history of malignancy, or a history of HIV infection. Patients with a history of recent antibiotic use or hospitalization (in the past 3 months) were also excluded. Diarrhea predominant IBS was defined as a patient who fulfilled the "Rome-IV criteria" for IBS and had recurrent abdominal pain on average for at least 1 day/week in the last 3 months, associated with two or more of the following: 1) improvement of abdominal pain after defecation; 2) change in frequency of stool passage (passing 3 stools/day with an average of 15 episodes/week for at least 3 months; 3) change in stool consistency or form. The objectives, safety, and data secrecy were highlighted to the study participants to obtain informed and written consent. The basic demographics like age, gender, duration of diarrhea, and weight were noted at the time of enrollment. Using a sterile syringe, 10 ml of venous blood was taken from every patient and dispatched to the institutional laboratory. Samples were centrifuged, and the serum was separated, dividing into two aliquots, which were immediately stored at -20 °C until analyzed in the laboratory. "Tissue transglutaminase antibodies (anti-TTG IgA and anti-TTG IgG)" were done for all patients in the laboratory and TTG positivity was noted. The Celikey ELISA analyzer by Phadia, Germany designed for automated detection of TTG antibodies using enzyme-linked immunosorbent assay (ELISA) technology was used. The process involved collecting and preparing serum samples, which were then loaded into wells of a pre-coated ELISA plate where anti-TTG antibodies, if present, bind to the TTG antigen. The analyzer operates under predefined settings optimized for the specific ELISA kits it uses, ensuring that the assay conditions are ideal for the detection of the specific antibodies. Unbound substances are washed away to reduce background noise. A secondary enzyme-conjugated antibody specific to human IgA or IgG is added and incubated. Another wash step removes unbound secondary antibodies. A chemi-luminescent substrate was added to the reaction. This substrate reacts with the enzyme on the secondary antibody to produce light. The emitted light is measured by a luminometers. The intensity of the light was quantified and converted into a numerical value representing the concentration of anti-TTG antibodies. Anti-TTG IgA or IgG antibody levels below 10 U/ml were considered negative, indicating no significant presence of these antibodies in the sample. Anti-TTG IgA or IgG antibody levels ≥ 10 U/ml were considered positive. An automated machine was used to ensure consistency and minimize human error. Anemia was labeled as hemoglobin below 12 g/dl among females, and <13 g/dl among males [8]. The data were analyzed using "IBM-SPSS Statistics", version 26.0. Age, duration of diarrhea, and weight were presented as mean and standard deviation (SD). For the representation of the qualitative variables like gender and TTG positivity, frequencies and percentages were computed. The TTG positivity was stratified among age, gender, duration of diarrhea, and weight. Post-stratification chi-square test was applied taking $p<0.05$ as significant.

RESULTS

In a total of 96 patients, 59 (61.5%) were male. The mean age, weight, and duration of diarrhea were 37.64±6.28 years, 81.48±7.70 kg, and 11.67±3.81 months, respectively. The residential status of 68 (70.8%) patients was rural. A family history of CD was noted among 13 (13.5%) patients. Anemia was identified in 28 (19.2%) patients. Weight loss of >5% in the past 6 months was reported by 36 (37.5%) patients (Table 1).

Table 1: Characteristics of Diarrhea Predominant IBS Patients (n=96)

Characteristics of Diarrhea Predominant IBS Patients (n=96)		Frequency (%)
Gender	Male	59 (61.5%)
	Female	37 (38.5%)
Age (years)	18-40	72 (75.0%)
	41-60	24 (25.0%)
Weight (kgs)	≤80	52 (54.2%)
	>80	44 (45.8%)
Duration of diarrhea (months)	3-6	61 (63.5%)
	>6	35 (36.5%)
Area of residence	Rural	68 (70.8%)
	Urban	28 (29.2%)
Family history of celiac disease	Yes	13 (13.5%)
	No	83 (86.5%)
Anemia	Yes	28 (29.2%)
	No	68 (70.8%)
Weight loss (>5% in the last 6 months)	Yes	36 (37.5%)
	No	60 (62.5%)
Bowel habits (times per day)	3-5	53 (55.2%)
	6-8	29 (30.2%)
	>8	14 (14.5%)
Presence of bloating	Yes	60 (62.5%)
	No	36 (37.5%)

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Duration of diarrhea above 6 months was found to have a significant linkage with TTG positivity (p<0.001). The TTG positivity in diarrhea-predominant IBS was seen to be in 12 (12.5%) patients (Figure 1).

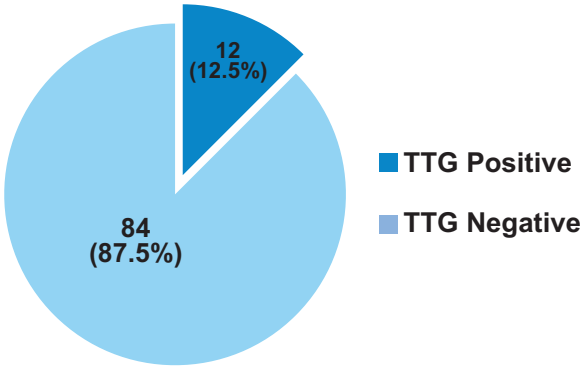

Figure 1: TTG Positivity in Diarrhea Predominant IBS (n=96)

No statistically significant association of TTG positivity was found with age groups (p=0.476), gender (p=0.812), and weight (p=0.757). Duration of disease above 6 weeks was found to have a significant association with TTG positivity (p<0.001) as all TTG-positive patients had a duration of disease above 6 months. Weight loss >5% in the past 6 months was significantly more among TTG positive patients (p=0.026). The stratification of TTG positivity concerning age, gender, and duration of diarrhea is shown (Table 2).

Table 2: Stratification of TTG Positivity in Diarrhea Predominant IBS Patients concerning characteristics of the patients (n=96)

Characteristics		TTG Positivity		p-value
		Yes (n=12)	No (n=84)	
Age (Years)	18-40	10 (83.3%)	62 (73.8%)	0.476
	41-60	2 (16.7%)	22 (26.2%)	
Gender	Male	7 (58.3%)	52 (61.9%)	0.812
	Female	5 (41.7%)	32 (38.1%)	
Duration of Diarrhea (Months)	3-6	-	61 (72.6%)	<0.001
	>6	12 (100%)	23 (27.4%)	
Weight (kg)	≤80	7 (58.3%)	45 (53.6%)	0.757
	>80	5 (41.7%)	39 (46.4%)	
Area of Residence	Rural	7 (58.3%)	61 (72.6%)	0.308
	Urban	5 (41.7%)	23 (27.4%)	
Family History of Celiac Disease	Yes	3 (25.0%)	10 (11.9%)	0.215
	No	9 (75.0%)	74 (88.1%)	
Anemia	Yes	6 (50.0%)	22 (26.2%)	0.090
	No	6 (50.0%)	62 (73.8%)	
Weight Loss (>5% in the Last 6 Months)	Yes	8 (66.7%)	28 (33.3%)	0.026
	No	4 (33.3%)	56 (66.7%)	
Bowel Habits (Times Per Day)	3-5	3 (25.0%)	50 (595%)	0.091
	6-8	5 (41.7%)	24 (28.6%)	
	>8	4 (33.3%)	10 (11.9%)	
Presence of Bloating	Yes	9 (75.0%)	51 (60.7%)	0.339
	No	3 (25.0%)	33 (39.3%)	

DISCUSSION

Understanding the frequency of TTG positivity in diarrhea-predominant IBS patients can have significant clinical implications as it may help in identifying potential cases of undiagnosed CD within the IBS population, guiding appropriate management strategies. In this study, 12.5% diarrhea predominant IBS patients tested positive for anti-TTG. This proportion (12.5%) is a little higher than what was reported by Mohammad et al from Iraq in 2019 where they described 5 out of 70 (7.1%) IBS patients with positive anti-TTG. Interestingly, out of those 5 five patients, 3 were diarrhea predominant IBS. The diarrhea predominant IBS patients must be screened for CD [9]. In the current study, as the patients were not subjected to duodenal biopsy & histopathology, it is difficult to report the exact prevalence of CD in IBS. Several studies in different regions have explored the burden of CD in IBS. In England, Sanders *et al.*, conducted a study with 300 IBS patients referred for secondary care, using Anti Gliadin Antibodies (AGA) and Endomysial Antibodies (EMA) as screening tests. They found that 4.7% of patients had CD confirmed by biopsy [10, 11]. Another study examining 1200 IBS patients revealed 3.3% of patients had duodenal biopsy findings compatible with CD [12]. A study from Jordan by Jadallah et al detected anti-TTG antibodies in 24 out of 742 IBS patients, with 3.23% confirmed to have CD through duodenal biopsies [13]. Hoseini-Asl *et al.*, from Iran, also reported a higher prevalence of CD in IBS, reaching 13.5% which is somewhat similar to what we noted [14]. A recent study by Al-Abachi from Iraq revealed that 10% of IBS patients were anti-TTG positive while 5% of these were confirmed biopsy-proven CD cases [15]. These studies collectively suggest that screening CD in IBS is advisable, especially when there is diarrhea predominant IBS of longer duration. An important finding in this study was that all 12 patients who tested positive for TTG experienced diarrhea for more than six months. This pattern can be explained by the chronic nature of CD, which typically presents with persistent and long-standing gastrointestinal symptoms [16]. Ongoing damage to the intestine in CD may impair nutrient absorption, resulting in chronic diarrhea and other gastrointestinal symptoms [17]. The extended duration of symptoms in CD contrasts with the often more episodic nature of diarrhea predominant IBS, where symptoms can fluctuate and may not be continuously severe [18]. The pathophysiology of CD involves a prolonged immune response to gluten, which gradually leads to the production of TTG antibodies. Therefore, the longer duration of diarrhea in TTG-positive patients is consistent with the progressive nature of CD and the time required for significant intestinal damage and antibody production to

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occur [19]. Studies have demonstrated that CD often presents with a longer history of symptoms before diagnosis. According to Lebwohl *et al,* patients with CD frequently report chronic diarrhea lasting several months to years before a diagnosis is made. This extended symptom duration may be necessary for the autoimmune response to gluten to manifest as elevated TTG antibodies [20]. Single study sites and the involvement of a modest sample size were some of the limitations of this study. We were unable to perform biopsy analysis for the confirmation of CD in this study which would have given us more valuable insights. Moreover, a full celiac serology panel including anti Deamidated gliadin peptide could have further illustrated seropositivity in IBS with diarrhea prominent patients.

CONCLUSIONS

It was concluded that the frequency of TTG positivity was high in diarrhea-predominant IBS patients. Screening for CD in IBS patients can be worth considering especially in cases with relatively longer duration of IBS symptoms.

Authors Contribution

Conceptualization: BK, AN, FN
Methodology: BK, SAK, SU
Formal analysis: FN, NU, SU
Writing, review and editing: BK, SAK, AN, FN, NU, SU

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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