Correlation of Tissue Transglutaminase Antibody with Duodenal Histologic Marsh Grading

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ABSTRACT

BACKGROUND

Recent guidelines have proposed that there is a correlation between tissue transglutaminase (tTG) antibody titers and degrees of duodenal biopsy, and that duodenal biopsy can be omitted in some patients with high levels of tTG antibody. Using data of registered patients in a gastrointestinal clinic we aimed to assess the correlation between tissue transglutaminase antibody with duodenal histologic Marsh grading in Iranian patients with celiac disease.

METHODS

We retrospectively reviewed hospital files of registered patients in the gastrointestinal clinic of Firoozgar Hospital, Tehran, Iran. Demographic, laboratory, and histology data of those who had tTG titer and pathology reports of duodenal biopsy based on the modified Marsh classification were extracted and used for the study.

RESULTS

159 patients with available tTG titer and pathology reports were enrolled in our study. Mean ±SD of the patients was 35.6±15.2 and 100 (62.9%) of them were women. 133 out of 153 patients had villous atrophy (Marsh IIIa-IIIc). Anemia was the most common sign and bloating, abdominal pain, and diarrhea were the first three common symptoms in these patients. Mean tTG titers was significantly higher in patients graded as Marsh III (p for trend=0.003). Our results showed that tTG titer more than 9 folds higher than the kit's cut-off value was about 97.2% sensitive for Marsh II and more duodenal damage.

CONCLUSION

There was a correlation between tTG titers and degrees of duodenal damage in patients with celiac disease. Duodenal biopsy is not always necessary for diagnosing celiac disease and when tTG level is more than 9 folds higher than the manufacture's recommended cut-off value it can be avoided. Meanwhile small intestinal biopsy should always be considered in case of high clinical suspicion, regardless of the results of serologic testing.

KEYWORDS
Celiac disease;Tissue Transglutaminase Antibody; Histology; Marsh Grading

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INTRODUCTION

According to Oslo's definition in 2013, "celiac disease is a chronic small intestinal immune-mediated enteropathy precipitated by expo-

sure to dietary gluten in genetically predisposed individuals".[1] A definite diagnosis of celiac disease is based on histological changes, including intraepithelial lymphocytosis, crypt hyperplasia, and varying degrees of villous atrophy, graded according to a classification system proposed by Marsh (Marsh I-IIIc).[2]

Recently developed screening tests such as anti-endomysial (AEA) and specially tissue transglutaminase (tTG) antibodies have remarkably improved the diagnosis rate and screening programs for celiac disease. Broad utilization of these tests have helped indicate that celiac disease is more prevalent than what we previously thought[1,2] and it is not an exclusively gastrointestinal disease and can be presented by a variety of clinical presentations.[3-7]

The tendency towards using non-invasive and less expensive methods for the diagnosis of celiac disease, especially in children, has prompted researchers to check if there is any correlation between tTG levels and mucosal damage and whether it has sufficient positive predictive value (PPV) to be solely used for the diagnosis of celiac disease. Recent evidence has shown that histological duodenal changes are correlated with tTG titers,[8-10] and proposed that duodenal biopsy can even be ignored in strongly positive tTG levels with some additional symptoms and history.[11-13]

The prevalence of celiac disease in Iran has been reported to be high (1 out of every 104 healthy subjects) and tTG test is wildly available and used in our setting for celiac disease screening. Therefore, using the serologic tests for diagnosis of celiac disease, at least in a specific group of patients, can be cost effective. We aimed to check whether degrees of mucosal damage to the small bowel correlate with clinical presentation and serum markers of Iranian patients with celiac disease.

MATERIALS AND METHODS

By reviewing records of the outpatient gastrointestinal clinic of Firoozgar Hospital, affiliated to Iran University of Medical Sciences, Tehran, Iran, documents of 159 patients who had been evaluated for celiac disease for any reason and had tTG titer and pathology reports of duodenum were enrolled. Endoscopy procedure and pathology review had been performed by experienced gastroenterologists and a single trained pathologist. Pathology reports from duodenal biopsies had been reported according to the modified Marsh classification. Anti-tTG IgA antibody along with serum IgA antibody were tested for all patients in our hospital's laboratory using an enzyme-linked immunosorbent assay (ELISA) technique by a commercially available kit (ORG540 A, ORGENTEC Diagnostica GmbH). Antibody levels above 10 Au/ml were considered positive, as per the manufacturers' recommended tTG cut-off value. Laboratory data including complete blood count (CBC), liver, and thyroid function tests, ferritin, iron, TIBC, and calcium levels along with demographic data, clinical symptoms, and medical history of all the patients were extracted from their hospital records. If any information was missed, the patients were contacted by phone and were asked for any available data. The study protocol was approved by the Ethics Committee of Iran University of Medical Sciences and informed consent was obtained from all the patients after explaining the aims and protocol of the study. Statistical analysis was done using STATA package and $p<0.05$ was considered as statistically significant.

RESULTS

The study population included 59 (37.1%) men and 100 (62.9%) women. The mean±SD age of the patients was 35.6±15.2 years (range: 3.5-78 years). The demographic characteristics of the participants is shown in table 1.

As shown in table 1, the overall prevalence of anemia in patients with villous atrophy was 72.9% and anemia was the most common non-gastrointestinal (GI) sign. However we could not find any significant association between Marsh grading and hemoglobin levels (p for trend=0.55).

Although the frequency of most GI symptoms was higher in "Marsh IIIc" than other Marsh grading, there were no statistically significance differences in GI symptoms between different Marsh grading. Our results showed that while there was no

Table 1: Frequency (%) and mean±SD of some descriptive characteristics of the patients in different Marsh staging

Variable		Modified Marsh Classification					VA+ Marsh>II
		Normal & Marsh I	II	IIIa	IIIb	IIIc	
Frequency (%)		13 (8.2)	13 (8.2)	55 (34.6)	41 (25.8)	37 (23.2)	133
Mean ±SD age (year)		39.1±17.5	35.9±10.7	33.1±16.9	36.5±14.2	37.1±14.4	35.2 (15.4)
Sex	Female	10 (76.9)	6 (46.2)	34 (61.8)	28 (68.3)	22 (59.5)	84 (63.2)
	Male	3 (23.1)	7 (53.8)	21 (38.2)	13 (31.7)	15 (40.5)	49 (36.8)
Anemia		7 (53.8)	8 (61.5)	39 (70.9)	29 (70.7)	27 (73.0)	98 (73.7)
Bloating		3 (23.1)	9 (69.2)	35 (63.6)	27 (65.8)	25 (67.5)	87 (65.4)
Abdominal pain		5 (38.4)	5 (38.4)	31 (56.3)	22 (53.6)	24 (64.8)	77 (57.8)
Diarrhea		6 (46.1)	4 (30.7)	25 (45.4)	22 (53.6)	22 (59.4)	69 (51.8)
Weight loss		8 (61.5)	4 (30.7)	24 (43.6)	18 (43.9)	21 (56.7)	63 (47.3)
Fatigue		5 (38.4)	3 (23.1)	25 (45.4)	9 (21.9)	15 (40.5)	49 (36.8)
Anorexia		2 (15.3)	4 (30.7)	18 (33.3)	7 (17.5)	15 (40.5)	40 (30.5)
Aphthous		2 (15.3)	4 (30.7)	16 (29.1)	8 (19.5)	11 (29.7)	35 (26.3)
Constipation		3 (23.1)	4 (30.7)	13 (23.6)	10 (24.3)	10 (27.1)	33 (24.8)
Osteoporosis		0	0	8 (14.8)	3 (7.5)	5 (13.5)	40 (30.5)

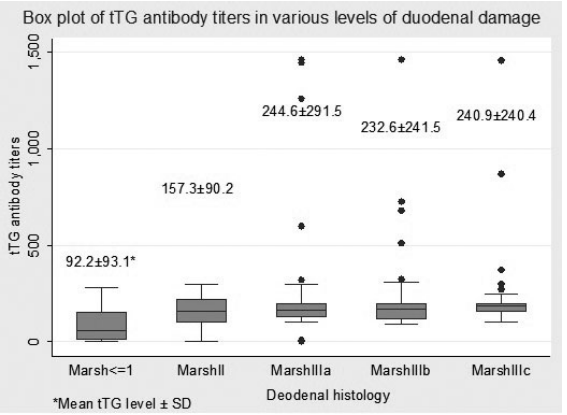

Fig. 1: Tissue transglutaminase (tTG) antibody titers in various levels of duodenal damage.

significant differences in tTG levels in patients with villous atrophy, mean tTG titers were significantly lower in Marsh I and II grades than the other three grades (*p* for trend= 0.33 and 0.003 respectively). Figure 1 shows the increasing trend of mean tTG antibody titers from normal duodenal histology to complete atrophy.

Receiver-operator curve (ROC) analysis was used to find a cut-off point for tTG antibody to discriminate A: absence (Marsh≤II) vs presence (Marsh IIIa-c) of villous atrophy and B: Marsh 0-I vs Marsh II_III. Figure 2 represents these two ROC curve graphs. While area under curve (AUC) for graph A was poor; AUC=0.67 (Std. error 0.068 and 95% CI 0.53-0.80); for graph B it is almost acceptable; AUC= 0.76 (Std. error 0.086 and 95% CI 0.59-0.93). Optimal cut-off points and corresponding sensitivity and specificities were calculated according to Youden index (J) =maximum {sensitivity – specificity - 1}.

DISCUSSION

Our study showed that there was a correlation between tTG levels and degrees of duodenal damage. And tTG levels more than 9 times the manufacturers' recommended cut-off value (tTGA≥90 U/ml) was about 97.2% sensitive for Marsh II and more duodenal damage. These results are in agreement with recent findings.[10,12,14]

Prior to the adventure of tissue transglutaminase (tTG) antibody, antigliadin antibody and endomysial antibody (EMA) have been used as serologic tests for diagnosis and screening of celiac disease. Although the specificity of EMA was very high but inadequate sensitivity of these tests resulted in some seronegative celiac cases, making them undesirable in clinical practice[15] TTG antibody was recognized by Dieterich and colleagues in 1997 as the major endomysial autoantigen and has been used as

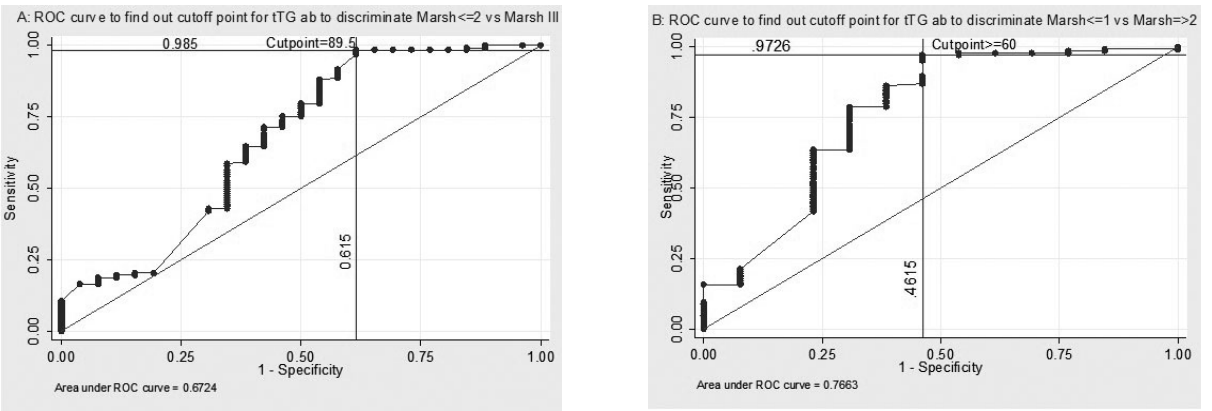

Fig. 2: ROC curve analysis to find out cut-off point for tTG ab to discriminate two different standard A: Marsh <=1 vs Marsh=>2; B: Marsh <=2 vs Marsh IIIa-c.

a sensitive and specific ELISA based test in celiac disease[16-18] Following this identification, during the previous decade, studies have shown that tTG levels are different in various degrees of intestinal damage and there is a correlation between titers of tTG antibody and marsh grading.[8-13,19,20]

In 2005, when tTG had still not broadly been used in clinical settings, Barker and co-workers proposed that for subgroup of pediatric patients with celiac disease with very high tTG titers small-bowel biopsy was not necessary to make the diagnosis[21] Later Vivas and colleagues showed that duodenal biopsy might be avoided in children with strongly positive tTG antibody titers.[11] European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) for Diagnosis of Celiac Disease have updated their guideline on the diagnosis of celiac disease after 20 years, and have mentioned that duodenal biopsy can be omitted from the diagnostic process of celiac disease in patients with anti-TG2 titers more than ten times the ULN and confirmatory positive EMA and HLA testing.[13] This diagnostic approach has been confirmed by Mubarak and co-workers, that small intestinal biopsy can be avoided in symptomatic patients with tTGA≥100 U/ml[12] Recently Allesio and colleagues, have concluded that, in both adults and children, there is a high probability of duodenal damage in patients with positive anti-tTG serology ≥ 7 times the cut-off, along with positive EMA, and under specific conditions duodenal biopsy could be avoided for the diagnosis of celiac disease[10] Zanini and co-workers have even proposed that more than 5 times the upper limit of the normal level is 100% specific for duodenal atrophy and can be used as cut-off, which could help avoid biopsy in one third of adult patients with celiac disease[14] As mentioned earlier, we found that tTG levels more than 90 U/ml were about 97.2% sensitive for Marsh II and more duodenal damage.

Moreover, it has also been shown that tTG antibody levels were significantly lower in patients who strictly adhered to a gluten free diet than those who did not[22] Initial evaluation of celiac disease (CD) was based on a combination of positive CD-specific serological tests and histological findings in the intestinal biopsy. TTG antibody is used to initially screen suspected, including symptomatic and asymptomatic individuals for celiac disease and to monitor adherence and response to gluten free diet.[23]

Results of our study also showed that anemia was the most common sign and bloating, abdominal pain, and diarrhea were the first three common symptoms in patients with celiac. These results are in agreement with the results of a recent study.[24]

Most of the studied patients had Marsh IIIa to IIIc grades in pathology. The small number of patients with Marsh I and II grading was one of main limitations of our study. Furthermore, the EMA re-

sults of most of our patients were not available and we could not use these data in our analysis.

Considering the high prevalence of celiac disease in Iran, which has been reported to be 1:104[25] similar to western countries,[1,26] it seems that tTG test can be applied for diagnosis of celiac disease, without duodenal biopsy, at least in patients with very high titers of serum antibody, between 5 to 10 folds than normal values. Confirmatory EMA and supporting clinical evidence should also be considered. In spite of this finding, small intestinal biopsy should always be considered in case of high clinical suspicion, regardless of the results of serologic testing.[5]

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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