



## Correlation Between Cut-off Level of Tissue Transglutaminase Antibody and Marsh Classification

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### ABSTRACT

#### BACKGROUND

Duodenal biopsy is required for diagnosis of celiac disease in adults, although some studies have suggested adequate accuracy of serology alone.

**Objective:** We aimed to assess the correlation between anti-tissue transglutaminase (tTG) titer and pathological findings and to define the specific level of tTG for predicting celiac disease in adults without the need for biopsy sampling.

#### METHODS

This descriptive study was done on 299 participants. The tTG titer and pathological findings of duodenal biopsy samples were used for this study. Analysis of Receiver operating characteristic (ROC) curve was used to find a cut-off point of anti-tTG antibody for mucosal atrophy.

#### RESULTS

Mean tTG titers was significantly higher in patients graded as Marsh III  $\geq$  3 ( $p=0.023$ ). ROC curve analysis showed 89.1% sensitivity for cut-off point  $\geq$  76.5 IU/mL of anti-tTG. For Marsh  $\geq$  II, specificity was 28% and positive predictive value was 91%.

#### CONCLUSION

There is a linear correlation between increasing tTG level and Marsh I to III. Specificity of tTG titer more than 200 was 100% for Marsh  $>$ 2.

**KEYWORDS:** Celiac Disease, Tissue transglutaminase antibody, Diagnosis, Pathology

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### INTRODUCTION

Celiac disease (CD) is a gluten related autoimmune disease occurring in genetically susceptible patients. Patients with CD show a wide variety of clinical manifestations.<sup>1</sup> The diagnosis of CD is based on clinical findings, serological tests, and histopathological evaluations.<sup>2,3</sup> The first paraclinical step in the diagnosis of CD is serological assessment. There are several serological antibodies for early detection.<sup>1,4</sup> There is no reliable cut-off level of serology in adult CD by now. Initial evaluation of CD is based on detecting tissue transglutaminase immunoglobulin (IgA) antibodies.<sup>2-6</sup> There are several studies suggesting that

there is a correlation between immunoglobulin serological titers and the degree of villous abnormalities in the gastrointestinal (GI) tract.<sup>7-11</sup> Previous studies showed anti-tTG level more than 100 had high specificity for Marsh III, and titer of 2 to 14 times of kit references had high PPV for mucosal atrophy in CD.<sup>8,9,12</sup>

The European Society of Pediatric Gastroenterology and Nutrition (ESPGAN)<sup>12</sup> in its recent guidelines considered the diagnosis of CD in children without any biopsies and only by clinical manifestations and serological tests. Serological evaluations and specifying certain cut-off levels for immunoglobulin titers may also be useful in adult patients.<sup>13-16</sup> Marcis and colleagues showed that there was a possibility of diagnosing CD without invasive endoscopy and biopsy sampling by considering specific level of serology.<sup>17</sup>

Some studies have demonstrated a linear relationship between anti-tTG levels and villous abnormalities in Marsh grade I up to Marsh grade III C, and duodenal biopsy can be prevented in suspected patients having CD related clinical manifestations and relevant history with a strongly positive anti-tTG level.<sup>8,10</sup>

According to the fact that the treatment of CD is gluten free diet for the whole patient's life, the diagnosis should be considered on serology, just if the false positive results are approximately zero. The aim of our study was to assess the correlation between anti-tTG titers and Marsh classification in north-east Iran and to find out a reliable cut-off level with acceptable specificity for predicting mucosal atrophy without the need for biopsy sampling.

## MATERIALS AND METHODS

### Study protocol and pathological examination:

In this study, 299 seropositive patients with CD who aged more than 12 years, and had pathology more than Marsh I that were referred to the celiac disease center from 2010 to 2014 were enrolled. The tTG assay was performed by enzyme-linked immunosorbent assay (ELISA). The kit (Euroimmune, Germany) was used in one research laboratory and results >20(IU/mL) were considered as positive (manufacture's cut-off value >20 IU/mL as positive). Although there is a considerable variability of commercially ELISA kits supplied by different manu-

factures, we worked on the fold rise of normal tTG titer too.

All the patients had undergone endoscopic evaluation and at least four biopsy samples of the duodenum had been taken. Pathological evaluation of the duodenum was reported by a single expert GI pathologist based on the modified Marsh classification.<sup>18</sup>

### Data collection:

This was a retrospective study on our collected data including age, sex, clinical presentation, anti-tTG level, and modified March classification score.

### Ethical considerations:

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences and informed consents were obtained from the participants.

### Statistical analysis:

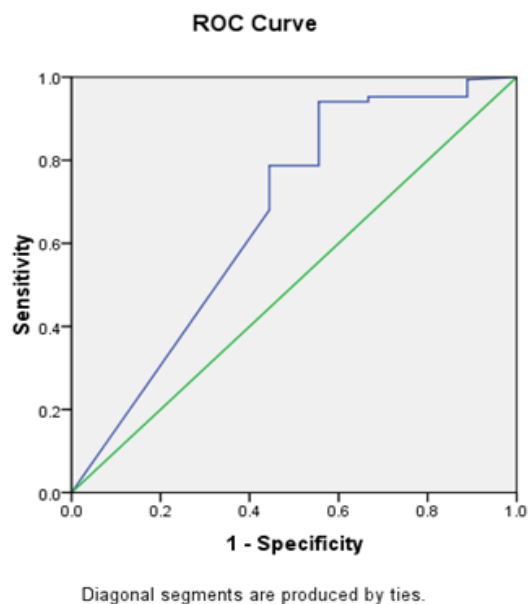
The data were analyzed using SPSS software, version 16.<sup>5</sup> (SPSS Inc., Chicago, IL, USA). Descriptive statistics (mean, standard deviation, and relative frequency) were used to describe and to summarize the basic characteristics of the patients. Afterwards, ROC curve and Pearson test were used. P values <0.05 were considered as statistically significant.

## RESULTS

The mean age of the participants was 33.0±13.6 years (range: 12-76 years) and 69.9% (214 individuals) were female.

Our results showed that while there were significant differences in tTG titer in CD, mean tTG titers in Marsh I was 120±73 (IL/mL), in Marsh II was 157.5±62.3(IL/mL) and in Marsh III was 178.6±44.3(IL/mL) based on Oberhuber's classification<sup>20</sup>. Based on modified Marsh classification<sup>19</sup>, 3a was 147±60.8(IL/mL), 3b was 160.9±58.9(IL/mL), and in Marsh 3c was 182.5±38.5(IL/mL) with significant difference (p=0.001). Mean tTG titers in all patients were 172±51.

ROC characteristic analysis was used for identifying



**Fig.1:** Receiver operating curve demonstrating the maximum area under the curve for Marsh III & II histopathology at anti-tTG titer of 76.5 IU/mL.

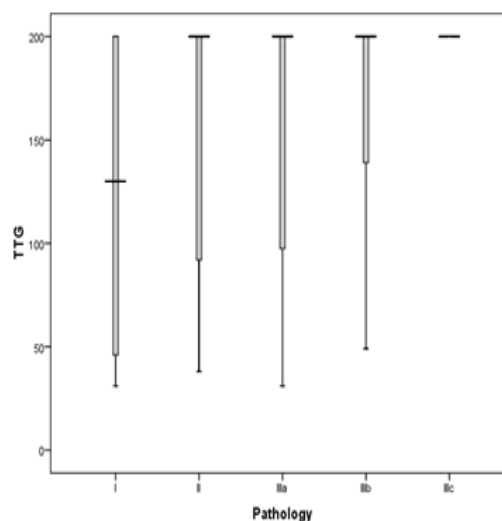
the cut-off point of anti-tTG antibody as a marker for mucosal atrophy. As it can be seen in figure 1, for anti-tTG levels more than 76.5 U/mL, ROC curve analysis had the highest area under the curve (AUC) in the presence of Marsh II and III with a sensitivity of 89%, specificity of 28%, positive predictive value (PPV) of 91%, and a negative predictive value (NPV) of 37% (AUC=59: 95% CI=48-70).

Although an increase in the antibody titer cut-off level may increase the specificity and PPV, it may also decrease the AUC and the test sensitivity. Optimal cut-off points and corresponding sensitivity and specificities were calculated according to Youden index ( $J$ )=maximum (sensitivity+specificity-1).

As it can be seen, there is an approximate linear correlation between anti-tTG levels and the severity of villous abnormalities in the GI tract from Marsh grade I up to grade III C (figure 2).

Considering the folding increase of anti-tTG titer, we have different sensitivity and specificity for having higher grade of atrophy. Increase in fold rise up to 10 times of normal limit cause 100% specificity of anti-tTG for mucosal atrophy (table 1).

Finally, we could not identify any correlation between the anti-tTG titer and the disease clinical manifestations.



**Fig.2:** Serum tTG antibody level vs. Marsh classification.

## DISCUSSION

This study was done to determine the cut-off point of anti-tTG for mucosal atrophy in CD in north-east Iran. In our study, we detected a significant correlation between anti-tTG titer and the degree of GI tract mucosal atrophy. Present study also showed,  $tTG \geq 200$  IU/mL was 100% specific for Marsh III.

Previous studies have suggested that in symptomatic patients duodenal biopsy can be avoided if anti-tTG level is more than 100 U/mL (kit value of  $>10$  as positive).<sup>9</sup> Value of 10 times of normal limit was associated with villous atrophy of the GI tract mucosa<sup>21-22</sup> and more severe clinical presentations with sensitivity and specificity of 98% and 99%, respectively.<sup>8</sup>

In a study by Fernández-Bañares and colleagues, tTG titer of at least 11.4 times of normal had a PPV of 98.6%.<sup>22</sup> In all these studies the researchers found that more than 10 times of normal level for anti-tTG in adults could be diagnostic for villus atrophy as it is in children. In our study 93% of the patients with Marsh III had anti-tTG more than 76 (IU/mL) and 100% of the patients with anti  $tTG \geq 200$  (IU/mL) (10 times of normal value) had Marsh III.

In a study by Emami and co-workers, sensitivity of

**Table 1: Sensitivity and specificity of IgA anti-tTG (by titer and fold rise) in patients with Marsh scores  $\geq$ II and Marsh III**

IgA anti-tTG	For Marsh score $\geq$ II and III						
	8 (2.7%)	12(4%)	9(3%)	13(4.3%)	7 (2.3%)	91(30%)	159(53%)
Number of patients (%)	8 (2.7%)	12(4%)	9(3%)	13(4.3%)	7 (2.3%)	91(30%)	159(53%)
Anti-tTG titer (fold-rise)	40(2)	60(3)	80(4)	100(5)	120(6)	140(7)	200(10)
Sensitivity for villous abnormality (modified Marsh grade $\geq$ II) (%)	96.4	93.5	89.9	83.4	79.9	78	68
Sensitivity for villous abnormality (modified Marsh III)	97.8	95.2	92.9	88.5	85.9	83.6	73
Specificity for villous abnormality (modified Marsh grade $\geq$ II)	12	45	45	45	45	56	100
Specificity for villous abnormality (modified Marsh III)	6	23	28.6	32.6	35.3	38.1	100

tTG for diagnosis of CD with Marsh III C was 80%.<sup>23</sup> In previous study they found higher specificity and PPV for predicting Marsh III with increasing the titer and in ROC analysis, the highest curve proportion was achieved exactly at 62.5 U/mL of anti-tTG titer with a sensitivity, specificity, PPV, and NPV of 95.4%, 98%, 93.8%, and 91.3%, respectively for predicting Marsh  $\geq$ III.<sup>24</sup> In another study that was performed on 159 patients with CD in Iran, 9 times of normal anti-tTG level had 97% sensitivity for Marsh  $\geq$ II.<sup>25</sup>

In our ROC curve analysis for Marsh II and III, the highest curve proportion was achieved at 76.5 U/mL of anti-tTG level with a sensitivity of 89%, PPV of 91%, and NPV of 37%. So anti-tTG (IgA) level  $\geq$ 76.5 IU/mL predict higher degree of villous atrophy. In our study tTG level  $\geq$ 200 IU/mL (10 times of normal kit value) was about 100% specific for Marsh III. Our finding is in agreement with previous studies. With increasing titer of serology we have an increase in the specificity and PPV for diagnosis of mucosal atrophy and CD. So, low level of positive serology cannot be diagnostic for mucosal atrophy. We also found that there was a linear correlation between Marsh I to III and tTG level by Spearman correlation ( $r=0.58$   $p=0.04$ ). In contrast with the results of Dahlbom and colleagues,<sup>8</sup> our experiment showed that classical and non-classical presentations had no correlation with tTG titer.

There is an increasing PPV for diagnosis of CD by increasing the anti-tTG titer. There is also a linear correlation between increasing anti-TG level and degree of mucosal atrophy. CD can strongly be diagnosed with serology titer more than 76.5 IU/mL. Applying anti-tTG

(IgA) fold rise for definite diagnosis of CD, is more helpful. This novel study also points for further investigation of the role of tTG titer for accurate diagnosis of CD.

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