http:// ijp.mums.ac.ir
Original Article (Pages: 15076-15082)

Serum Anti TTG Level as a Predictor for Severity of Iintestinal Damage in Children with Celiac Disease

Seyed Ali Jafari[1], Maryam Khalesi[1], Hamidreza Kianifar[1] , Hoda Shojai[2], *Mohammad Ali Kiani[1]

[1] Department of Pediatric Gastroenterology, Akbar Medical Center, Mashhad University of Medical Sciences, Mashhad, Iran.

[2] Department of pediatrics, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Background: This study aimed at evaluating how the patient's clinical manifestations and his/her anti TTG level are correlated with the intensity of histological damage based on the classification of Marsh Oberhuber.

Methods: This cross-sectional study was performed on 186 children suspected of celiac disease who referred to gastroenterology clinics between 2014 and 2015 and had Anti TTG >20. All patients underwent upper gastrointestinal endoscopy and multiple biopsies of duodenum were taken; histological classification was performed based on Marsh Oberhuber criteria. Finally, the relationship between serum levels of Anti TTG and histologic findings was assessed based on Marsh Oberhuber criteria.

Results: There was a statistically significant difference between Anti TTG and different classes of grading March (P=0.01). Moreover, the intensity of intestinal damage based on Marsh Oberhuber criteria was significantly higher in children who had gastrointestinal complaints, the classic form of celiac disease and growth disorder. Anti TTG level of 148 IU/ml was the best positive cutoff point suggested by the ROC. For anti TTG levels of 148 IU/ml, sensitivity, specificity, positive predictive value and negative predictive value were 46.8%, 82.4%, 91.7%, and 27.2%, respectively.

Conclusion: Anti TTG level of 148 IU/ml has a high positive predictive value and a low negative predictive value for histologic changes more than grade 1 in Marsh classification.

Key Words: Celiac disease, Intestinal damage, Serum Anti-TTG level.

* Please cite this article as: Jafari A, Khalesi M, Kianifar H, Shojai H, Kiani MA. Serum Anti TTG level as a predictor for severity of intestinal damage in children with celiac disease. Int J Pediatr 2021; 9 (12):15076-15082. DOI: **10.22038/IJP.2021.59273.4619**

**** Corresponding Author:***

Mohammad Ali Kiani, Department of Ppediatric Gastroenterology, Akbar Medical Center, Mashhad University of Medical Sciences, Mashhad, Iran. Email: Kianima@mums.ac.ir

Received date: Jul.26,2021; Accepted date:Aug.31,2021

1- INTRODUCTION

Celiac disease is a chronic, small intestine immune-mediated disease which occurs with receiving gluten in genetically susceptible patients. It is a common disease with prevalence of 0.5 to 1% in most of the general populations (1). The first step for the diagnosis of celiac disease in suspected patients is serological evaluations. There were multiple serological tests for screening celiac disease. The most valuable test is IgA anti-tissue transglutaminase antibody (anti TTG-IgA) with a high sensitivity and specificity compared to other antibody tests (2, 3). Patients with a positive anti TTG-IgA should undergo intestinal biopsy for the definite diagnosis of celiac disease based on histologic findings (4-9). According to the modified Marsh classification, the histological features of CD were classified as grade 1 with an increase in intraepithelial lymphocyte, grade 2 with crypt hyperplasia but no villous flattening and grade 3 with villous atrophy. Although there are some studies suggesting histological confirmation for all patients with positive serologic tests, but others suggest that intestinal biopsy can be optional for patients with typical gastrointestinal symptoms of celiac disease and high levels of anti TTG-IgA over 10 times more than the normal upper limit (10-15).

This study, thus, aimed at evaluating how the patient's clinical manifestations and his/her anti TTG level are correlated with the intensity of histological damage based on the classification of Marsh Oberhuber.

2- MATERIALS AND METHODS

This cross-sectional study was carried out in Ghaem Medical Center of Mashhad University of Medical Sciences, Iran during a 24-month period (March 2015-March 2017). The protocol for this study was approved by the Ethics Committee of Mashhad University. Consecutive series of patients less than 18 years old suspected to have celiac disease with serum anti TTG level >10 (IU/ML) on gluten contained diet, who were referred to gastroenterology clinic were enrolled. Anti TTG level was measured with Diametra kit (product of Italy) by ELISA method in the laboratory of Dr. Sheikh hospital. Patients with any of the following criteria were considered as suspected for celiac disease and underwent the evaluation of serum Anti TTG level. According to the test symptoms suggesting celiac disease (intestinal and extra intestinal), high risk patients according to the screening panel for celiac disease, and hyper transaminasemia with unknown etiology were identified. Upper gastrointestinal endoscopy was performed for all patients and multiple biopsies (4 species from the second part of duodenum and at least one species from duodenal bulb) were taken. All biopsy species were evaluated by a single experienced gastro-intestinal pathologist who was blinded to the original evaluation, clinical, and laboratory data. Mucosal changes in each slide were scored using the Marsh criteria as modified by Oberhuber (I=increased intraepithelial lymphocytes; II=increased intraepithelial lymphocytes and crypt hyperplasia; III = increased intraepithelial lymphocytes, crypt hyperplasia and villous atrophy). If multiple changes were present within a single fragment or series of fragments in one patient, the most severe lesion was recorded.

Patients with IgA deficiency and patients on gluten free diet or suspected of having started the gluten free diet before the histological evaluation, patients whose parents didn't permit for performing endoscopy, and patients with normal histologic findings were excluded. The patients' data including demographics, clinical manifestations, laboratory results (complete blood count, blood urea nitrogen, creatinine, alanine aminotransferase, and aspartate

aminotransferase), and histologic findings were recorded. After entering data into SPSS version 16.0 software, quantitative data were analyzed by the Wilcoxon signed rank test and qualitative data by the McNemar test. A *P* value < 0.05 was considered significant. Post-estimation analyses of receiver operating curve (ROC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used to predict cutoff points for Anti TTG titers as predictors of grades higher than 1 in Marsh classification.

3- RESULTS

A total of 186 patients with positive anti-TTG-IgA tests were, initially, enrolled in the study. Among them 14 patients were excluded due to IgA deficiency (4 patients), initiation of a gluten free diet before performing endoscopy (5 patients) and not permitting us to perform endoscopy (5 patients). Among 172 patients who underwent endoscopy and biopsy, 80 (46.5%) cases were females. Mean age of the patients was 7.7±3.5 years. Mean serum anti TTG level was 175.54±128.66 IU/ML. The most common histologic grading was Marsh 3 with a frequency of 58.7% (101 patients) followed by mash 2 (22.0%, 38 patients) and Marsh 1 (19.1%, 33 patients).

The mean z score of weight was -1.26±1.35 and the mean z score of height was -0.92±1.36. Patient's characteristics, according to the histologic grading, were summarized in **Table 1**. There was no significant difference in age, sex, weight and height Z score among three histological groups. Poor growth and gastrointestinal manifestations (abdominal pain, diarrhea, and constipation) were significantly more common presentations in patients with Marsh 3 histological grading.

Table-1: Baseline characteristics of the patients according to Marsh histologic classification

Baseline characteristics	Marsh histologic classification				P-value
	Grade 0	Grade 1	Grade 2	Grade 3	
Age, year (Mean ± SD)	7.93 ± 4.5	8.73 ± 3.06	8.71 ± 3.11	7.27 ± 3.41	0.17
Sex(Female/Male)	2/1	38/35	22/31	28/23	0.4
Growth index					
Weight Z-score (Mean ± SD)	-0.95 ± 1.03	-0.99 ± 1.2	-1.12 ± 1.0	-1.4 ± 1.46	0.24
Height Z-score (Mean ± SD)	-0.73 ± 1.2	-0.81 ± 1.4	-0.64 ± 1.1	-1.07 ± 1.38	0.21
Etiology of evaluation					
Poor growth	0	7	12	46	0.001
Gastrointestinal symptoms	0	3	8	22	0.001
Anemia	0	0	1	2	-
Edema	0	0	0	1	-
Hypertransaminasemia	0	1	1	3	-
Turner syndrome	1	0	0	1	-
Celiac disease in sibling	2	1	1	2	0.44
Diabetes mellitus	0	19	15	24	0.43

Means of the different anti TTG levels according to Marsh grading were shown in **Table 2**. Serum anti TTG level was significantly higher in patients with Marsh 3 histological grading (P- value=0.01)

Ordinal logistic regression showed that there are significant and direct relationships between increased levels of Anti TTG and severity of pathological damages according to Marsh grading

(P<0.001). Anti TTG level of 148 IU/ml was the best positive cutoff point suggested by the receiver operating curve. For anti TTG levels of 148 IU/ml, sensitivity, specificity, positive predictive value and negative predictive value were 46.8%, 82.4%, 91.7%, and 27.2%, respectively. The area under the curve was determined as 67.7% (CI 74% - 95.6%).

Table-2: Anti TTG levels according to Marsh histologic classification

Histologic classification	Anti TTG-IgA(Mean ± SD)	P-value
Marsh grade 0	67.92 ± 93.13	0.01
Marsh grade 1	107.52 ± 83.14	
Marsh grade 2	149.14 ± 114.81	
Marsh grade 3	202.03 ± 147.74	

4- DISCUSSION

In our study, mean anti TTG was significantly higher in patients with higher grades of Marsh histologic findings. Anti TTG titer of 148 IU/mL (fourteen times higher than the limit of normality) was a good predictor for Marsh grade 2 or higher grades with a relatively high specificity and positive predictive value of 82.4 %, 91.7%, respectively.

Although histology is considered the standard diagnostic test for the diagnosis of celiac disease, its invasive nature is the major limitation. In addition, samples must be properly positioned, oriented, and sectioned. Incorrect preparation of the sample can simulate a false diagnosis of celiac disease. So availability of noninvasive serologic tests has improved the diagnostic accuracy and has emphasized the limits of histological examination (16-18).

Several studies have investigated the correlations between autoantibody levels and the extent of mucosal damage, with the aim of limiting the number of endoscopies required for the diagnosis of celiac disease. Singh et al. have reported that the positive predictive value of anti TTG 14-fold more than normal is about 100%; and the increase of less than 2 times in anti TTG is associated with Marsh levels more than 2. However, despite this finding, they have concluded that bowel biopsy should be done, even if the increase is less than 2 times in the level of antibody. This conclusion was due to the fact that there were multiple patients with low antibody titers who had celiac disease according to histopathologic results (19). Mubarak et al. reported a specificity of 38.2% for Anti TTG level of 47 IU/ml. Their suggestion was that among symptomatic patients, duodenal biopsy can be avoided in Anti TTG > 100 IU/ml (the kit positive value was more than 10); their study on 183 children revealed that Anti TTG has a positive predictive value of > 100 is 100%, specificity of 100%, negative predictive value of 93% and sensitivity of 97% (20). Fernardez-Banares et al. reported that an anti TTG titer of at least 11.4% times more than normal has a predictive value of 98.6% (21). In another study by Alessio et al. on 412 patients with the mean age of 10 to 72 years, an anti TTG 7 times more than normal was associated with significant mucosal damage (Marsh > 2) independent of age and sex with the specificity and predictive value of 100%. Anti TTG 20 times more than normal had the specificity of > 99.8% to predict the patients with villous atrophy (Marsh c, b, 3a) (22).

In opposition to several studies that suggest high diagnostic accuracy of serologic tests for celiac disease, there were multiple studies with different results. Elitsu et al. evaluated 240 children

with suspected celiac disease. They reported that the sensitivity, specificity, positive predictive value and negative predictive value of anti TTG 10 times more than normal were 75.4%, 48.8%, 87.7%, and 29%, respectively (23). Hawamdeh et al. reported that the sensitivity, specificity, positive predictive value, and negative predictive value of anti TTG 180 IU/ml were 81.6%, 56.6%, 78.4% and 70%, respectively (24). Ganji et al. in a study on 299 adult patients with suspected celiac disease reported that sensitivity, specificity, positive predictive value and negative predictive value of anti TTG level of 76.5 IU/ml were 89%, 28%, 91%, and 37%, respectively. They found that the classical and non-classical presentation of disease have no relationship with intestinal histologic change, while in our study, the patients referred with gastrointestinal complaints or growth disorder had significantly higher intensity of intestinal damage ($P<0.001$) (25).

Due to the need to obtain four to six samples from various locations in duodenal biopsy for the diagnosis of celiac disease, it is possible that the difference between the results of different studies be due to different accuracies in obtaining biopsy specimens and histopathological interpreting of biopsy species or different statistical societies in the studies (adult patients and pediatric patients). But it seems that the most important factor explaining the differences is the consideration of different cut off points for Anti TTG level as the prognostic factor for histological changes. It will be strengthened in the absence of international reference values of Anti TTG level (26, 27). European Society of Gastroenterology, Hepatology and Nutrition suggests an increased antibody of 10 times more than normal for avoiding biopsy samples in suspected patients (28). As in many laboratories, Anti TTG levels above 10 IU/ml are considered as normal, an upper limit level of 100 IU/ml can be considered as a cut off point for Anti TTG level. It is important to define an accurate cut off point for prevention of excessive uses of endoscopic procedures, so that almost none of the patients with celiac would need performing endoscopy.

In our study, the Anti TTG level of 148 IU/ml had sensitivity, specificity, positive predictive value and negative predictive value of 46.8%, 82.4%, 91.7%, and 27.2%, respectively. It has, thus, a high positive predictive value and a low negative predictive value for prediction of histologic changes more than grade 1 of Marsh classification. Previously, patients with Anti TTG levels between 100 to 148 IU/ml were considered as appropriate cases for non performing endoscopy. However, considering that there were multiple false negative results, the presently found cutoff point suggests that a higher level of Anti TTG should be considered for this purpose.

5- CONCLUSION

Results of the present study suggest that the Anti TTG level of 148 IU/ml enjoys a high positive predictive value and a low negative predictive value for prediction of histologic changes more than grade 1 of Marsh classification. Despite our previous method of considering patients with Anti TTG levels between 100 to 148 IU/ml as appropriate cases for non performing endoscopy, considering the previously mentioned multiple false negative results, we suggest considering a higher level of Anti TTG level for this purpose.

6- CONFLICT OF INTERESTS STATEMENT: None

7- ACKNOWLEDGMENTS

The authors appreciate the cooperation of Nooshin Abdollahpour, who provided technical help. Also, thanks go to Dr.

MohamadReza Zirak for his help in analyzing the research data.

8- REFERENCES

1. Rubio-Tapia A, Hill ID, Kelly CP, et al. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol 2013; 108:656.

2. Snyder J, Butzner JD, DeFelice AR, et al. Evidence-Informed Expert Recommendations for the Management of Celiac Disease in Children. Pediatrics 2016; 138.

3. Leffler D, Schuppan D, Pallav K, et al. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. Gut 2013; 62:996.

4. Kurppa K, Salminiemi J, Ukkola A, et al. Utility of the new ESPGHAN criteria for the diagnosis of celiac disease in at-risk groups. J Pediatr Gastroenterol Nutr 2012; 54:387.

5. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012; 54:136.

6. Stewart MJ, Shaffer E, Urbanski SJ, et al. The association between celiac disease and eosinophilic esophagitis in children and adults. BMC Gastroenterol 2013; 13:96.

7. Zanini B, Magni A, Caselani F, Lanzarotto F, Carabellese N, Villanacci V, Ricci C, Lanzini A. High tissue-transglutaminase antibody level predicts small intestinal villous atrophy in adult patients at high risk of celiac disease. Digestive and Liver Disease. 2012 Apr 30; 44(4):280-5.

8. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. European journal of gastroenterology & hepatology. 1999 Oct 1; 11(10):1185.

9. Aldaghi MA, Dehghani SM, Haghighat M. Evaluation of the correlation between tTG-IgA titer and duodenal biopsy findings in children with suspected celiac disease. Iranian journal of pediatrics. 2016 Feb; 26(1).

10. Emami MH, Karimi S, Kouhestani S. Is routine duodenal biopsy necessary for the detection of celiac disease in patients presenting with iron deficiency anemia? International journal of preventive medicine. 2012 Apr 1; 3(4).

11. Bhattacharya M, Lomash A, Sakhuja P, Dubey AP, Kapoor S. Clinical and histopathological correlation of duodenal biopsy with IgA anti-tissue transglutaminase titers in children with celiac disease. Indian Journal of Gastroenterology. 2014 Jul 1; 33(4):350-4.

12. Beltran L, Koenig M, Egner W, Howard M, Butt A, Austin MR, Patel D, Sanderson RR, Goubet S, Saleh F, Lavender J. High-titre circulating tissue transglutaminase-2 antibodies predict small bowel villous atrophy, but decision cut-off limits must be locally validated. Clinical & Experimental Immunology. 2014 May 1; 176(2):190-8.

13. Dahlbom I, Korponay-Szabo IR, Kovács JB, Szalai Z, Mäki M, Hansson T. Prediction of clinical and mucosal severity of coeliac disease and dermatitis herpetiformis by quantification of IgA/IgG serum antibodies to tissue transglutaminase. Journal of pediatric gastroenterology and nutrition. 2010 Feb 1; 50(2):140-6.

14. Hill ID, Dirks MH, Liptak GS, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2005; 40:1.

15. Khalesi, M. and Jafari, S. A. and Kiani, M. and Picarelli, A. and Borghini, R. and Sadeghi, R. and Eghtedar, A. and Ayatollahi, H. and Kianifar, H. R. In vitro gluten challenge test for celiac disease diagnosis. Journal of Pediatric Gastroenterology and Nutrition, 62 (2). pp. 276-283.

16. Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, Fasano A. Celiac disease: a comprehensive current review.2019 Jul 23;17(1):142.

17. Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, Murray JA, Verdu EF, Kaukinen K. Coeliac disease. Nat Rev Dis Primers. 2019 Jan 10; 5(1):3.

18. Gallegos C, Merkel R. Current Evidence in the Diagnosis and Treatment of Children with Celiac Disease. Gastroenterol Nurs. 2019 Jan/Feb; 42(1):41-48.

19. Singh P, Kurray L, Agnihotri A, Das P, Verma AK, Sreenivas V, Dattagupta S, Makharia GK. Titers of anti-tissue transglutaminase antibodies correlate well with severity of villous abnormalities in celiac disease. Journal of clinical gastroenterology. 2015 Mar 1; 49(3):212-

20. Mubarak A, Wolters VM, Gmelig-Meyling FH, Ten Kate FJ, Houwen RH. Tissue transglutaminase levels above 100 U/mL and celiac disease: a prospective study. World J Gastroenterol. 2012 Aug 28; 18(32):4399-03.

21. Fernández-Bañares F, Alsina M, Modolell I, Andújar X, Piqueras M, García-Puig R, Martín B, Rosinach M, Salas A, Viver JM, Esteve M. Are positive serum-IgA-tissue-transglutaminase antibodies enough to diagnose coeliac disease without a small bowel biopsy? Post-test probability of coeliac disease. Journal of Crohn's and Colitis. 2012 Sep 1; 6(8):861-6.

22. Alessio MG, Tonutti E, Brusca I, Radice A, Licini L, Sonzogni A, Florena A, Schiaffino E, Marus W, Sulfaro S, Villalta D. Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. Journal of pediatric gastroenterology and nutrition. 2012 Jul 1; 55(1):44-9.

23. Elitsur Y, Sigman T, Watkins R, Porto AF, Puppa EL, Foglio EJ, Preston DL. Tissue Transglutaminase Levels Are Not Sufficient to Diagnose Celiac Disease in North American Practices Without Intestinal Biopsies. Digestive Diseases and Sciences. 2017 Jan 1; 62(1):175-9.

24. Hawamdeh H, Al-Zoubi B, Al Sharqi Y, Qasrawi A, Abdelaziz Y, Barbar M. Association of Tissue Transglutaminase Antibody Titer with Duodenal Histological Changes in Children with Celiac Disease. Gastroenterology Research and Practice. 2016 Oct 27; 2016.

25. Ganji A, Esmaeilzadeh A, Bahari A, Ghafarzadegan K, Aghayee MA, Mozafari HM. Cut Off level of Tissue Transglutaminase Antibody for Diagnosing Celiac Disease in Adults. Middle East J Dig Dis. 2016; 8:318-22.

26. Bibbò S, Pes GM, Dore MP. Coeliac disease from pathogenesis to clinical practice: current concepts. Recenti Prog Med. 2020 Feb; 111(2):91-101. doi: 10.1701/3309.32799.

27. Alhassan E, Yadav A, Kelly CP, Mukherjee R. Novel Non-dietary Therapies for Celiac Disease. Cell Mol Gastroenterol Hepatol. 2019; 8(3):335-345.

28. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012; 54:136.