Middle East J Dig Dis. October 2023;15(4):263-269

doi 10.34172/mejdd.2023.356 http://mejdd.org

Middle East Journal of Digestive Diseases MEJDD

Original Article

Do Serological Tests Eliminate the Need for Endoscopic Biopsy for the Diagnosis of Symptomatic Patients with Celiac Disease? A Retrospective Study with Review of Literature

Mohammad Hossein Anbardar[1], Neda Soleimani[1], Ehsan Torabi Dashtaki[1], Naser Honar[2], Mozhgan Zahmatkeshan[2], Sahand Mohammadzadeh[1*]

[1]Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Celiac disease is one of the most common genetic allergies worldwide. The prevalence of celiac disease in Iran is similar to or even higher than the global prevalence. Celiac disease is a chronic inflammatory disease that affects the small intestine. Affected patients are allergic to gluten protein that exists in some grains, such as wheat and barley.

Methods: Serological endomysial IgA antibody (EMA-AB) and tissue transglutaminase IgA antibody (TTG-IgA) tests were performed on 114 patients aged the ages of 0–18 years with histopathological findings of celiac disease. The results of these tests were compared to the results of the histopathological study of the duodenal biopsy.

Results: Based on the receiver operating characteristic (ROC) curve and a calculation of the TTG-IgA test's sensitivity and specificity, the best diagnostic limit for the TTG-IgA test is 144, which has the best sensitivity and specificity. At this value (cut-off), the test's sensitivity was 62%, and the specificity was 93.7%. For the endomysial test, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 80%, 93%, 90%, and 75%, respectively.

Conclusion: The diagnostic accuracy of the endomysial test is better than that of the TTG-IgA test in general for diagnosing patients with celiac disease. In the TTG-IgA test, false-positive cases are high due to a cut-off of 20, reducing the test's specificity. In these false-positive cases, the endomysial test helps in better diagnosis.

Keywords: Celiac disease, EMA-AB, TTG-IgA

Cite this article as: Anbardar MH, Soleimani N, Torabi Dashtaki E, Honar N, Zahmatkeshan M, Mohammadzadeh S. Do serological tests eliminate the need for endoscopic biopsy for the diagnosis of symptomatic patients with celiac disease? A retrospective study with review of literature. *Middle East J Dig Dis* 2023;15(4):263-269. doi: 10.34172/mejdd.2023.356.

Received: February 10, 2023, **Accepted:** August 7, 2023, **ePublished:** October 30, 2023

Introduction

Celiac disease is a chronic inflammatory disease that affects the small intestine. The affected patients are sensitive to a protein called gluten, found in some cereals, such as wheat, barley, rye, etc. It is one of the most common food allergies worldwide, affecting approximately 1% of the world's population. Most cases of celiac disease are not diagnosed.[1-4]

In celiac disease, the gold standard for diagnosis is the histopathological study of the sample obtained from endoscopy and biopsy of the early part of the small intestine, usually the duodenum.[5,6] Two samples are taken from each area. The best locations are the bulb and the second part of the duodenum.

Microscopic findings show a range of changes in the epithelial surface and structure of the intestinal mucosa.[7,8] So, celiac disease is not the only disease with microscopic signs. Other diseases may exhibit the same histopathological signs. Therefore, in order to diagnose celiac, we often need the clinical signs, laboratory results, genetic tests, histopathological findings, and finally, the patient's response to a gluten-free diet to be consistent as pieces of a puzzle.[9] As was already said, the gold standards for diagnosing celiac disease are an endoscopy and a biopsy of the small intestine.

However, endoscopy is an expensive and invasive examination and requires general anesthesia in children, with associated risks. In addition, screening is recommended in susceptible populations, such as first-degree family members with celiac disease and those with type I diabetes and autoimmune thyroid disease. As a result, effective screening tests that can identify suitable endoscopic candidates are needed. The European Society of Gastroenterology, Hepatology, and Pediatric Nutrition has also remarked that serological tests can diagnose celiac disease without a biopsy.[10,11] Therefore, patients with celiac disease can avoid the risks of this invasive test, the cost of general anesthesia, and other costs by using serological tests and doing less endoscopy and sampling.[12,13]

By looking at the antibodies to endomysial IgA antibody (EMA-AB) and tissue transglutaminase IgA antibody (TTG-IgA) in the serum of people who might have celiac

*Corresponding Author: Sahand Mohammadzadeh, Email: mohammads@sums.ac.ir

disease, we hope to compare the results of small bowel biopsy, which is the gold standard for diagnosing celiac disease, with the results of these tests and figure out how sensitive and specific they are.

The role of EMA and TTG-IgA in diagnosing celiac disease is well established in previous studies.[14,15] The goal of this study was to determine whether the positive results of these two tests will be able to correctly diagnose the disease in people who are likely to have celiac disease based on clinical findings and eliminate the need for endoscopy. We calculate the sensitivity and specificity of this test in quantities of test results and, if possible, determine which cut-off of the test results is possible to diagnose celiac correctly. We also want to determine if there is a link between the amount of antibody titer in the TTG-AB test and the amount of mucosal damage in the biopsy.

Materials and Methods

Patient Selection

This study was a retrospective cross-sectional study that compared the diagnostic power of the TTG test with the ELISA method and the results of the EMA test with the indirect immunofluorescent method in the diagnosis of celiac disease. Children aged 0 to 18 years who had signs of celiac disease and were referred to Nemazee hospital and Motahari clinic in Shiraz were included in the study.

Inclusion Criteria

Inclusion criteria were typical clinical signs and symptoms of celiac disease, including diarrhea, weight loss, fatigue, abdominal pain, nausea, and vomiting. Due to changes in the expression of antibodies in people treated for celiac, patients should not have been on a gluten-free diet before.

Exclusion Criteria

Cases whose microscopic examination of the biopsy specimen was suspicious or undetectable, and it was not possible to rule out or confirm celiac disease based on pathology, were excluded from the study. If the biopsy result was mild and at the level of Marsh 1 and 2, considering that similar pathology in these people may be found in other diseases and conditions, these patients were also excluded. IgA-deficient patients were also excluded from the study due to the possibility of negative tests.

Sampling Method

Patients were visited by a pediatric gastroenterologist, and endoscopy was requested due to the signs and symptoms of celiac disease, along with elevated or normal TTG-IgA levels. A sample was taken from the patients during a routine visit to the endoscopy room. The amount of TTG-IgA and EMA-AB in the patient's serum was measured from this sample. The patient's form, including name, age, TTG-IgA level, and signs and symptoms of patients referred by a gastroenterologist, was completed. Test serum should be clear and non-hemolyzed.

Description of the Experiment

We used the indirect immunofluorescence tests from the EUROIMMUN kit (Germany) to perform an indirect immunofluorescence test to find IgA antibodies against endomysial tissue. Positive and negative controls are needed to ensure that the test steps are performed correctly and that the mixtures and solutions used in the test are safe. For positive control, we used a mixture containing human antibodies against endomysial tissue, and for the negative control, we used a mixture without these antibodies. When adding the patient's serum to the slides, these mixtures should be in contact with the monkey's esophageal tissue instead of the patient's serum. Both mixes were provided by the kit manufacturer. Fluorescent-labeled antibodies find endomysial antibodies that bind to the endomysium tissue of smooth muscle in the sample to make a specific fluorescence pattern. Based on the fluorescence intensity seen and the kit brochure, the test result can be announced relatively quantitatively. The level of staining was graded (strong reaction) using a scale ranging from zero (no reaction) to three (strong reaction) (i.e., + 1 (weak reaction), + 2 (moderate reaction)).

TTG-IgA was measured by AESKULISA ELISA kit (Germany). Human recombinant tissue transglutaminase is bound to microwells. The determination is based on an indirect enzyme-linked immune reaction. Specific antibodies in the patient's sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently, the added enzyme conjugate binds to the immobilized antibody-antigen complexes. After incubation, a second washing step removes unbound enzyme conjugate. After adding the substrate solution, the bound enzyme conjugate hydrolyzes the substrate, forming a blue-colored product. The addition of an acid stops the reaction, generating a yellow end-product. The yellow color's intensity correlates with the antibody-antigen-complex concentration and can be measured photometrically at 450 nm. The reference amount announced by kit:

- Negative: Less than 20 u/mL
- Positive: Equal or more than 20 u/mL

Pathological Examination

All patients' slides were taken out of the file, evaluated by a pathologist, and re-graded using the Marsh criteria. Histological findings in this study define celiac disease. The Marsh type 3 lesion has three subgroups: 3a mild villous atrophy and pathological increase of intraepithelial lymphocytes (IEL); 3b moderate villous atrophy and pathological increase of IEL; 3c total villous atrophy and pathological increase of IEL.

Statistical Analysis

The SPSS software version 23 was used to analyze the

data, and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of EMA-AB and TTG-IgA were calculated using the receiver operating characteristic (ROC) curve.

Results

The study population included 114 children aged 0 to 18 years with symptoms of celiac disease who were referred to Nemazee hospital and Motahari clinic in Shiraz. The mean age of the patients was 8.8 years, and the standard deviation was 6.7. The average age of female patients was 9.2 years with a standard deviation of 7.6, and the mean age of male patients was 8.3 with a standard deviation of 7.6. A total of 49 (43%) children had histopathological changes of celiac disease in the biopsy, and 65 (57%) did not have such changes in duodenal histology. In terms of sex, 49 (43%) were boys, and 65 (57%) were girls. In the celiac group, the ratio of girls to boys was 1.8 (32 girls and 17 boys), and in patients without the disease, this ratio was 1.06 (33 girls and 32 boys).

Histopathological Findings

Among patients with celiac disease, 15 (13%) had Marsh 3a, 26 (23%) had Marsh 3b, and 8 (7%) had Marsh 3c. Of these patients, 37 showed two microscopic changes in the bulb and D2. Regarding this group of patients, where there is a difference between the degree of celiac disease in the two parts, D1 and D2, we diagnosed based on the higher grade of the disease.

TTG-IgA Levels

Based on the information in the kit, values of less than 20 were normal, and those of 20 or above were positive. In this study, the range of TTG-IgA obtained was 0-436, with an average of 60.

In our study, the number of false-positive TTG-IgA cases (values greater than 20 without pathology in the duodenum) was 48 (42.1%). Of these 48 cases, four patients had an EMA-AB test (+1), and the number of false-negative cases (values less than 20 with pathology in the duodenum) was five. In all these false negatives, the amount of TTG-IgA was less than 17. Of these five cases, two patients had a positive EMA-AB test (+1).

The one-way analysis of variance (*P* value: 0.05) showed that increasing the amount of TTG-IgA from normal to celiac is significantly related. However, there was no clear link between the levels of antibodies and the severity of the disease in the Marsh 3a, 3b, and 3c celiac groups.

Quantitative values for each level of TTG values are given in Table 1. For each level of identification of TTG values and sensitivity and specificity, the number of PPV and NPV was calculated. According to this curve and also Table 1 of sensitivity and specificity, the best diagnostic limit for the TTG-IgA test is 144, in which there is the best specificity against the best sensitivity. At this value (cut-off), the sensitivity of the test was 62%, and the specificity of the test was 93.7%.

Table 1. Quantitative diagnostic values for each level of TTG cut-off

TTG Result	Sensitivity	Specificity	Positive predictive value	Negative predictive value
6	100.00	17.19	48.5	100.0
17	90.00	23.44	47.9	75.0
41	84.00	56.25	60.0	81.8
45	80.00	57.81	59.7	78.7
60	74.00	70.31	66.1	77.6
85	66.00	79.69	71.7	75.0
100	64.00	85.94	78.0	75.3
144	63.00	93.7	88.6	75.9
200	50.00	96.87	92.6	71.3
259	46.00	100.00	100.0	70.3

EMA-AB Values

According to the information in the kit brochure, the positive result of the kit is shown as the presence of a special fluorescence design in the form of a beehive under the texture of the mucosa layer, and the mucosa layer, which lacks fluorescence, is black (Figure 1).

For the endomysial test, sensitivity, specificity, PPV, and NPV were 80%, 93%, 90%, and 75%, respectively (Table 2). In an indirect immunofluorescence study, out of 65 people without celiac disease, four had a false-positive result (+1), but no higher amount of fluorescent was seen in these patients. Two of these patients were female, and their TTG test results were 295 and 139. The results of TTG for the other two male patients were 215 and 73. False-negative results were seen in nine patients. Among these people, six had a positive result for the TTG test, and among these, only two cases had TTG values higher than the cut-off value of 144 and TTG values higher than 340.

Among those with severe disease (3a), three cases had a negative indirect immunofluorescence test, and nine, two, and one cases showed +1, +2, and +3 results. Among those with the 3b stage of celiac disease, five cases had a negative result, two cases showed a +1 result, 16 out of the 26 patients with grade 3b had a positive result (+2), and three showed a +3 result. Among those with grade 3a, one case showed a negative result in indirect immunofluorescence. The result of +1 was not seen; one person had a result of +2, and six patients showed a +3 result.

The diagnostic accuracy of both EMA and TTG tests together in the diagnosis of celiac disease

In all patients, if we consider the EMA and TTG IgA cut-off test 144, patients with either an EMA-AB or TTG-AB positive result are considered to have celiac disease, and those with both tests or one test negative are considered to have celiac disease, the sensitivity will be 93.8%, specificity 93.8%, PPV 93.8%, and NPV 93.8% (Table 3).

Discussion

Celiac disease is a distinct gastrointestinal disorder

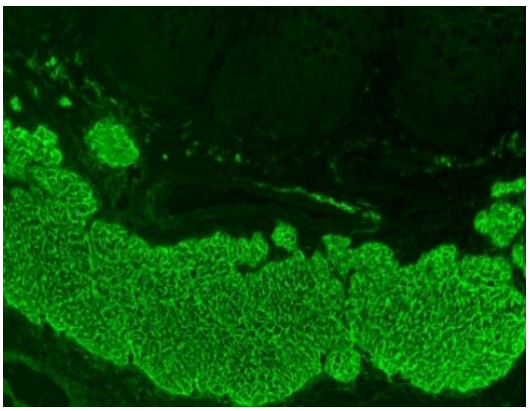

Figure 1. Honeycomb-like fluorescence pattern for EMA-IgA along muscolaris mucosa of monkey esophagus

because, apart from its clinical and pathophysiological characteristics, sensitive and specific serological markers allow its diagnosis. A significant milestone in the history of celiac disease was the discovery of the EMA serological biomarker for the disease in 1980. In 1997, Dieterich and colleagues identified the transglutaminase 2 autoantibody that targets endomysial antibodies. Then, autoantibodies to tissue transglutaminase were introduced by ELISA with high sensitivity and specificity, and as a result, celiac disease was recognized as a common global disease.[16]

This study was performed on children aged 0 to 18 years, suspected of having celiac disease, who were referred to the endoscopy department of Motahari Clinic by a pediatric gastroenterologist. These people were clinically suspected of having celiac disease.

Regarding the EMA test, the sensitivity was 80%, the specificity was 93%, and a significant relationship was found between the severity of the disease based on the pathology and the amount of fluorescent observed in the test. Also, regarding TTG-IgA, according to the ROC curve, for calculating the sensitivity and specificity, the best diagnostic limit for the TTG-IgA test is 144, in which there is the best specificity against the best sensitivity. At this value (cut-off), the test's sensitivity was 62%, and the specificity of the test was 93.7%.

Although the TTG test is widely used by laboratories as a first-line due to its high sensitivity and repeatability, false-positive TTG tests are usually seen in low values of the titer of this antibody and in titrations up to about twice the diagnostic cut-off. Therefore, due to false positive results that are relatively common due to the high specificity of reverse immunofluorescence for the detection of EMA antibodies, the EMA test for patients with suspected celiac disease is often performed as a confirmatory test before biopsy in individuals who have a TTG-positive test.[17] The sensitivity and specificity of the TTG test in the diagnosis of celiac disease when the EMA test is also positive is about 100%.[18] Reverse immunofluorescence detection causes problems for EMA antibody observation due to different levels of observer skill, interference with anti-nuclear or anti-smooth muscle antibodies, as well as different standards from laboratory to laboratory in interpreting

Table 2. Sensitivity, specificity, positive predictive value, and negative predictive value for IgA endomysial test

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
EMA-AB	%80	93.75%	90.9%	75.7%

Table 3. Sensitivity, specificity, positive predictive value, and negative predictive value for EMA- IgA and TTG-AB

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
EMA-AB and TTG-AB	%93.8	93.8%	93.8%	93.8%

EMA testing. Also, there are ethical questions about how the endomysial tissue bed from the monkey's esophagus is prepared.[15]

In our study, with increasing disease severity, the mean Marsh as well as the upper and lower limits of the TTG test result, increase to grade 3b in proportion to the severity of the disease, but these values decrease in more severe diseases (Marsh 3c).

TTG test results in other studies[19-25] show different sensitivity and specificity values. Table 4 shows that even though the PPV of the TTG test varies and is sometimes low (between 70% and 99%), In our study, the sensitivity and specificity stood at 63% and 93.7%, respectively. The specificity is close to one, but the inconsistency between the sensitivities might be related to the difference in the volume of samples, the sensitivity of kits, or the difference in the stage of the disease.

De Chaisemartin et al in 2015[26] assessed 100 patients with celiac disease who were under treatment and showed that endomysial testing was best associated with villi atrophy and Marsh grading compared with TTG-IgA and TTG-IgG and a few lesser-known serological markers. In this study, the cut-off was 17.9, with a sensitivity of 53.1 and a specificity of 96.2.

Ganji and colleagues conducted a study in northeast Iran and found a linear relationship between increased TTG-AB titers and disease severity. The highest mean TTG-AB titer was seen among patients with Marsh 3. Also, based on the ROC curve, the TTG-AB test at a cut-off of 140 had a sensitivity of 83% and a specificity of 56%.[27]

In another study by Donaldson et al, the TTG-AB test was more than 98% specific for the diagnosis of celiac disease, and the EMA test with a titer above 1:1280 was more than 98% specific. They showed that higher TTG-AB values, as well as high EMA titers, were more likely to be associated with atrophy of the gastrointestinal villi.[28]

The results of the EMA test in other studies[29-34] show different sensitivity values ranging from 64% to 100%. The specificity of this test also varies from 93% to 100% (Table 5). In our study, the sensitivity and specificity stood at 80% and 93.7%. The specificity is close to one, but the inconsistency between the sensitivities might be related to the difference in the volume of samples, the sensitivity of kits, or the difference in the stage of the disease.

Based on the positive results of the EMA test and the

Table 4. TTG test results in other studies

Study	TTG					
	Test cut-off	Year	sensitivity	specificity	Positive predictive value	Negative predictive value
Abdollahi et al[19]	Not stated	2008	28%	95%	-	-
Alessio et al[20]	100 U/mL	2012	98%	97%	97.9%	98%
Lodhi et al [21]	25 U/mL	2017	91%	29%	76%	57%
Taneja et al[22]	84.6 U/mL	2021	91.7%	68.4%	94.2%	50%
Bansal et al[23]	70 U/mL	2018	83.9%	56.1%	86.8	50.2
Tortora et al[24]	62 U/mL	2014	69%	100%	100%	31%
Meena et al[25]	115 U/mL	2019	76%	100%	100%	17%
Our study	144 U/mL	2022	63%	93.7%	88.6%	75.9%

Table 5. The results of the EMA test in other studies

Study	EMA			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Abdollahi et al[19]	64%	96%	-	-
Carroccio et al[29]	100%	100%	100%	100%
Baudon et al[30]	90%	100%	100%	97%
Tesei et al[31]	86%	100%	100%	83%
Wolters et al[32]	92%	90%	-	-
Dickey et al[33]	80.8%	96.6%	-	-
Dahele et al[34]	86.8%	100%	-	-
Our study	80%	93%	90%	86%

severity of celiac disease, none of the people without celiac disease show fluorescence higher than + 2, and also, among people whose disease was grade 3a, 64% (9 out of 14 people) had a test of + 1. In cases with grade b3, this test was + 2 positive in 61% of patients (16 out of 26 people), and 75% of patients with severe disease (6 out of 8) showed + 3 positive results. So, in most cases, the severity of a positive test is related to how bad the disease is.

Serological screening for celiac disease is usually based on anti-TTG-IgA.[35,36] These antibodies are the most sensitive for celiac disease.[22] On the other hand, EmA-IgA is used to confirm a positive TTG test because it is more specific.[23]

False positives for TTG have been observed in patients with inflammatory bowel disease, food allergies, irritable bowel syndrome, anemia, giardiasis, other intestinal infections, and autoimmune disorders.[24-26] These false positives are not always addressable, as EMA test results are only reliable in laboratories with skilled personnel with experience in immunofluorescence assays. Commercial ELISA assays for TTG may vary depending on antigen quality, and there are differences between different kits in cut-off values. False negatives can be seen in celiac serological tests in children under two years of age, use of a gluten-free diet, IgA deficiency, use of corticosteroids, and laboratory error.[27,28]

The diagnostic accuracy of both the EMA and TTG tests together in the diagnosis of celiac disease increases in comparison with each test alone.[29] As seen in our results, the sensitivity and specificity increased. However, it does not eliminate the need for endoscopy, and patients with positive serology will still require a confirmatory endoscopy.

The current study has limitations, including a small sample size, an incomplete examination of patients' clinical symptoms, and the exclusion of asymptomatic patients. But there were some strengths, like looking at the samples' pathology, doing TTG and endomysial serological tests together, figuring out the cut-off based on the samples' pathology, and having patients of different ages, from 0 to 18 years.

Conclusion

According to the results, the sensitivity and specificity of the endomysial test are better than the TTG-IgA test to identify those individuals who require an intestinal biopsy examination to diagnose CD while avoiding unnecessary biopsy examinations in those who do not have the condition. In the TTG-AB test, false-positive cases are higher. In these cases, the endomysial test helps better diagnose, especially when the TTG-IgA is between 18 and 100, which includes false positives of TTG-IgA. It is better to check the endomysial antibody. Also, in cases where we are clinically suspected of having celiac disease but the TTG-IgA level is negative, checking the endomysial test has a high PPV. Finally, according to the obtained results, checking both of these tests for patients with suspected celiac disease will increase the diagnostic accuracy of serological tests to diagnose these patients. However, it does not eliminate the need for endoscopy, and patients with positive serology will still require a confirmatory endoscopy.

Authors' Contribution

Conceptualization: Mohammad Hossein Anbardar.
Data curation: Ehsan Torabi Dashtaki.
Formal analysis: Neda Soleimani.
Investigation: Neda Soleimani.
Methodology: Sahand Mohammadzadeh.
Project administration: Naser Honar.
Resources: Mozhgan Zahmatkeshan.
Software: Ehsan Torabi Dashtaki.

Supervision: Mohammad Hossein Anbardar.
Validation: Sahand Mohammadzadeh.
Visualization: Sahand Mohammadzadeh.
Writing–original draft: Sahand Mohammadzadeh.
Writing–review & editing: Sahand Mohammadzadeh.

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

Data Availability Statement
All data generated or analyzed during this study are included in this published article.

Ethical Approval
The research was conducted in accordance with the World Medical Association Declaration of Helsinki and approved by the Ethics Committee of Shiraz University of Medical Sciences (No. IR.SUMS.MED.REC.1398.628). Additionally, informed consent was obtained from the patients.

Funding
No funding was used in this study.

References
1. Catassi C, Fasano A. Celiac disease diagnosis: simple rules are better than complicated algorithms. *Am J Med* 2010;123(8):691-3. doi: 10.1016/j.amjmed.2010.02.019
2. Van Meensel B, Hiele M, Hoffman I, Vermeire S, Rutgeerts P, Geboes K, et al. Diagnostic accuracy of ten second-generation (human) tissue transglutaminase antibody assays in celiac disease. *Clin Chem* 2004;50(11):2125-35. doi: 10.1373/clinchem.2004.035832
3. Kurppa K, Ashorn M, Iltanen S, Koskinen LL, Saavalainen P, Koskinen O, et al. Celiac disease without villous atrophy in children: a prospective study. *J Pediatr* 2010;157(3):373-80, 80.e1. doi: 10.1016/j.jpeds.2010.02.070
4. Hadithi M, Peña AS. Current methods to diagnose the unresponsive and complicated forms of coeliac disease. *Eur J Intern Med* 2010;21(4):247-53. doi: 10.1016/j.ejim.2010.01.015
5. Sugai E, Nachman F, Váquez H, González A, Andrenacci P, Czech A, et al. Dynamics of celiac disease-specific serology after initiation of a gluten-free diet and use in the assessment of compliance with treatment. *Dig Liver Dis* 2010;42(5):352-8. doi: 10.1016/j.dld.2009.07.011
6. Copelton DA, Valle G. "You don't need a prescription to go gluten-free": the scientific self-diagnosis of celiac disease. *Soc Sci Med* 2009;69(4):623-31. doi: 10.1016/j.socscimed.2009.05.012
7. Dahle C, Hagman A, Ignatova S, Ström M. Antibodies against deamidated gliadin peptides identify adult coeliac disease patients negative for antibodies against endomysium and tissue transglutaminase. *Aliment Pharmacol Ther* 2010;32(2):254-60. doi: 10.1111/j.1365-2036.2010.04337.x
8. Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, et al. Celiac disease: a comprehensive current review. *BMC Med* 2019;17(1):142. doi: 10.1186/s12916-019-1380-z
9. Tye-Din JA, Galipeau HJ, Agardh D. Celiac disease: a review of current concepts in pathogenesis, prevention, and novel therapies. *Front Pediatr* 2018;6:350. doi: 10.3389/fped.2018.00350
10. Werkstetter KJ, Korponay-Szabó IR, Popp A, Villanacci V, Salemme M, Heilig G, et al. Accuracy in diagnosis of celiac disease without biopsies in clinical practice. *Gastroenterology* 2017;153(4):924-35. doi: 10.1053/j.gastro.2017.06.002
11. Sahin Y. Celiac disease in children: a review of the literature. *World J Clin Pediatr* 2021;10(4):53-71. doi: 10.5409/wjcp.v10.i4.53
12. Gould MJ, Brill H, Marcon MA, Munn NJ, Walsh CM. In screening for celiac disease, deamidated gliadin rarely predicts disease when tissue transglutaminase is normal. *J Pediatr Gastroenterol Nutr* 2019;68(1):20-5. doi: 10.1097/mpg.0000000000002109
13. Auricchio R, Mandile R, Del Vecchio MR, Scapaticci S, Galatola M, Maglio M, et al. Progression of celiac disease in children with antibodies against tissue transglutaminase and normal duodenal architecture. *Gastroenterology* 2019;157(2):413-20.e3. doi: 10.1053/j.gastro.2019.04.004
14. Elitsur Y, Sigman T, Watkins R, Porto AF, Leonard Puppa EL, Foglio EJ, et al. Tissue transglutaminase levels are not sufficient to diagnose celiac disease in North American practices without intestinal biopsies. *Dig Dis Sci* 2017;62(1):175-9. doi: 10.1007/s10620-016-4354-4
15. Wessels M, Velthuis A, van Lochem E, Duijndam E, Hoorweg-Nijman G, de Kruijff I, et al. Raising the cut-off level of anti-tissue transglutaminase antibodies to detect celiac disease reduces the number of small bowel biopsies in children with type 1 diabetes: a retrospective study. *J Pediatr* 2020;223:87-92.e1. doi: 10.1016/j.jpeds.2020.02.086
16. Korponay-Szabó IR, Dahlbom I, Laurila K, Koskinen S, Woolley N, Partanen J, et al. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. *Gut* 2003;52(11):1567-71. doi: 10.1136/gut.52.11.1567
17. Venugopal G, Mechenro J, Makharia G, Singh A, Pugazhendhi S, Balamurugan R, et al. Sequential testing with different tissue transglutaminase antibodies, a new approach for diagnosis of celiac disease. *Indian J Gastroenterol* 2017;36(6):481-6. doi: 10.1007/s12664-017-0803-z
18. Infantino M, Merone M, Manfredi M, Grossi V, Landini A, Alessio MG, et al. Positive tissue transglutaminase antibodies with negative endomysial antibodies: unresolved issues in diagnosing celiac disease. *J Immunol Methods* 2021;489:112910. doi: 10.1016/j.jim.2020.112910
19. Abdollahi A, Mehrazma M, Talachian E. The diagnostic value of serum IGG-antigliadin, IGA anti-endomysial and IGA anti-tissue transglutaminase antibodies for pediatric celiac disease. *Pediatr Res* 2011;70(5):775. doi: 10.1038/pr.2011.1000
20. Alessio MG, Tonutti E, Brusca I, Radice A, Licini L, Sonzogni A, et al. Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. *J Pediatr Gastroenterol Nutr* 2012;55(1):44-9. doi: 10.1097/MPG.0b013e3182470249
21. Lodhi MU, Stammann T, Kuzel AR, Syed IA, Ishtiaq R, Rahim M. Celiac disease and concomitant conditions: a case-based review. *Cureus* 2018;10(2):e2143. doi: 10.7759/cureus.2143
22. Taneja K, Mahajan N, Rai A, Malik S, Khatri A. Association of anti-tissue transglutaminase antibody titers and duodenal biopsy findings in pediatric patients of celiac disease. *Cureus* 2021;13(3):e13679. doi: 10.7759/cureus.13679
23. Bansal E, Kaur N, Mittal N. Can high titres of anti tissue transglutaminase antibodies reduce the need for intestinal biopsy for diagnosis of celiac disease? *Indian J Clin Biochem* 2018;33(4):456-60. doi: 10.1007/s12291-017-0695-9
24. Tortora R, Imperatore N, Capone P, De Palma GD, De Stefano G, Gerbino N, et al. The presence of anti-endomysial antibodies and the level of anti-tissue transglutaminases can be used to diagnose adult coeliac disease without duodenal biopsy. *Aliment Pharmacol Ther* 2014;40(10):1223-9. doi: 10.1111/apt.12970
25. Meena DK, Akunuri S, Meena P, Bhramer A, Sharma SD, Gupta R. Tissue transglutaminase antibody and its association with duodenal biopsy in diagnosis of pediatric celiac disease. *Pediatr Gastroenterol Hepatol Nutr* 2019;22(4):350-7. doi: 10.5223/pghn.2019.22.4.350
26. de Chaisemartin L, Meatchi T, Malamut G, Fernani-Oukil F,

Hosking F, Rault D, et al. Application of deamidated gliadin antibodies in the follow-up of treated celiac disease. *PLoS One* 2015;10(8):e0136745. doi: 10.1371/journal.pone.0136745
27. Ganji A, Esmaeilzadeh A, Bahari A, Ghafarzadegan K, Afzal Aghayee M, Mosanen Mozafari H, et al. Correlation between cut-off level of tissue transglutaminase antibody and marsh classification. *Middle East J Dig Dis* 2016;8(4):318-22. doi: 10.15171/mejdd.2016.42
28. Donaldson MR, Firth SD, Wimpee H, Leiferman KM, Zone JJ, Horsley W, et al. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clin Gastroenterol Hepatol* 2007;5(5):567-73. doi: 10.1016/j.cgh.2007.01.003
29. Carroccio A, Vitale G, Di Prima L, Chifari N, Napoli S, La Russa C, et al. Comparison of anti-transglutaminase ELISAs and an anti-endomysial antibody assay in the diagnosis of celiac disease: a prospective study. *Clin Chem* 2002;48(9):1546-50. doi: 10.1093/clinchem/48.9.1546
30. Baudon JJ, Johanet C, Absalon YB, Morgant G, Cabrol S, Mougenot JF. Diagnosing celiac disease: a comparison of human tissue transglutaminase antibodies with antigliadin and antiendomysium antibodies. *Arch Pediatr Adolesc Med* 2004;158(6):584-8. doi: 10.1001/archpedi.158.6.584
31. Tesei N, Sugai E, Vázquez H, Smecuol E, Niveloni S, Mazure R, et al. Antibodies to human recombinant tissue transglutaminase may detect coeliac disease patients undiagnosed by endomysial antibodies. *Aliment Pharmacol Ther* 2003;17(11):1415-23. doi: 10.1046/j.1365-2036.2003.01595.x
32. Wolters V, Vooijs-Moulaert AF, Burger H, Brooimans R, De Schryver J, Rijkers G, et al. Human tissue transglutaminase enzyme linked immunosorbent assay outperforms both the guinea pig based tissue transglutaminase assay and anti-endomysium antibodies when screening for coeliac disease. *Eur J Pediatr* 2002;161(5):284-7. doi: 10.1007/s00431-002-0933-9
33. Dickey W, McMillan SA, Hughes DF. Sensitivity of serum tissue transglutaminase antibodies for endomysial antibody positive and negative coeliac disease. *Scand J Gastroenterol* 2001;36(5):511-4. doi: 10.1080/003655201750153359
34. Dahele AV, Aldhous MC, Humphreys K, Ghosh S. Serum IgA tissue transglutaminase antibodies in coeliac disease and other gastrointestinal diseases. *QJM* 2001;94(4):195-205. doi: 10.1093/qjmed/94.4.195
35. Tonutti E, Visentini D, Bizzaro N, Caradonna M, Cerni L, Villalta D, et al. The role of antitissue transglutaminase assay for the diagnosis and monitoring of coeliac disease: a French-Italian multicentre study. *J Clin Pathol* 2003;56(5):389-93. doi: 10.1136/jcp.56.5.389
36. Suh-Lailam BB, Davis KW, Tebo AE. Immunoassays for the detection of IgA antibodies to tissue transglutaminase: significance of multiples of the upper limit of normal and inter-assay correlations. *Clin Chem Lab Med* 2016;54(2):257-64. doi: 10.1515/cclm-2015-0348