Is High Tissue Transglutaminase Antibody Titers Enough to Diagnose Celiac Disease in Children?

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Abstract

Background. The current standard method for diagnosis of celiac disease (CD) is an adequate small-bowel biopsy (usually obtained through upper gastrointestinal tract endoscopy) showing characteristic histopathological changes, followed by a therapeutic response to a gluten free diet. Commercially available IgA tissue trassglutaminase antibody (TTG) screening tests have been developed with variable sensitivities and specificities. The use of high titer cutoff values than currently recommended should improve the specificity of the test and its positive predictive value.

Objectives. To evaluate the significance of high TTG titers in the diagnosis of CD.

Methods. One hundred sixteen patients with signs and symptoms suggestive of CD had undergone TTG testings (IgA &IgG class) and small-bowel biopsies while 50 healthy volunteers without family history of CD, were sent for TTG testings only. Ten patients excluded from the study because their TTG values and small-bowel biopsies were negative.

Results. Ninety eight of 106 zpatients demonstrated positive biopsy results. Seventy two of 106 patients had IgA TTG levels of >100U/ml, with 70 of 72 exhibiting positive biopsy **Results.** Twenty two of 28 patients with IgA TTG values >18-100U/ml exhibited positive biopsy results. Six patients with negative IgA TTG levels of ≤18 U/ml had positive biopsies and positive IgG TTG levels (>18 U/ml).Two volunteers had positive IgA TTG levels (>18U/ml).The sensitivity and specificity of IgA TTG were 94.3% and 96% respectively while the sensitivity of duodenal biopsy was 92.6%(8 symptomatic patients had negative small-bowel biopsy while their IgA TTG values were positive).

Conclusions. Symptomatic patients with high titer TTG levels >100 U/ml can be treated as CD without small-bowel biopsy and a negative biopsy does not exclude CD.

Key words. Celiac Disease; Tissue Transglutaminase Antibody; Small-Bowel Biopsy.

Abbreviations. CD, Celiac Disease; TTG, Tissue Transglutaminase Antibody.

الخلاصة

المقدمه: الطرق القياسيه الحاليه في تشخيص مرض حساسيه الحنطه تتضمن اخذ نموذج كافي من النسيج الحي للامعاء الدقيقه(عاده من خلال ناظور للجزء الأعلى من القناة الهضميه) الذي يظهر تغيرات نسيجيه مميزه والذي يتبع باستجابه المريض للغذاء الخالي من الحنطه.ان التوفر التجاري لاختبار الغلوبين المناعي أ لنسيج الترانس كلوتامنيس الجسم المضاد كفحص غربله والذي اظهر نتائج مختلفه من الحساسيه والنوعيه.ان استعمال المعيار العالي من هذا الجسم المضاد وبشكل اوسع من استخدامه الحالي سيوفر دعم لنوعيه هذا الاختبار في اظهار نتائج ايجابيه معتمده.

الاهداف: لتقييم اهمية المعيار العالي من اختبار مضاد نسيج الترانس كليتامنيس في تشخيص مرض حساسيه الحنطه عند الاطفال.

الطرق:مئه وسته عشر مريض ممن يشكون من اعراض وعلامات متفقه مع مرض حساسيه الحنطه قد خضعوا لفحص مضاد نسيج الترانس كلوتامنيس مع اخذ نموذج من نسيج الامعاء الدقيقه بينما 50 شخص متطوع وبصحه جيده من الذين ليس لديهم تاريخ عائلي لمرض حساسيه الحنطه قد خضعوا لفحص الجسم المضاد فقط, 10 اشخاص قد اقصوا من البحث حيث ان كلا من نتائج فحص الجسم المضاد والنسيج كان سلبيا لديهم.

النتائج:ثمانيه وتسعون من 106 مريض اظهروا فحص نسيج ايجابي.اثنان وسبعون من 106 مريض كانت نتيجه اختبار الغلوبين المناعي أ لنسيج الترانس كلوتامنيس لديهم اكثر من 100 وحده/مليلتر حيث اظهر 70 من 72 منهم نتيجه فحص نسيج ايجابيه.اثنان وعشرون من 28 الذين يتراوح اختبار الجسم المضاد لديهم اكثر من 18-100 وحده/مليلتر اظهروا

فحص نسيج ايجابي.سته مرضى اظهروا نتيجه سلبيه لاختبار الغلوبين المناعي أ (حيث كانت نتيجه المعيار اقل من 18 وحده/مليلتر) جاءت نتيجه كلا من فحص النسيج واختبار الغلوبين المناعي ج (معيار اكثر من 18 وحده/مليلتر)ايجابيه بالنسبه لهم. اثنان من المتطوعين كان اختبار الغلوبين المناعي أ لنسيج الترانس كلونامنيس ايجابي(معيار اكثر من 18 وحده/مليلتر).ان حساسيه ونوعيه فحص الغلوبين المناعي أ لنسيج الترانس كلوتامنيس كانت 94.3%و96% تعاقبيا بينما حساسيه فحص نسيج الاثني عشري كانت 92.6%(8 مرضى ممن لديهم اعراض المرض اظهروا فحص نسيج سلبي في حين كانت نتائج الغلوبين المناعي أ لنسيج الترانس كلوتامنيس لجابه لديهم).

الاستنتاج:المصابين باعراض حساسيه الحنطه مع وجود معيار عالي من الجسم المضاد لنسيج الترانس كلوتامنيس يمكن ان يعالجوا كمرضى حساسيه الحنطه دون الحاجه لاخذ نسيج حي من الامعاء الدقيقه لان سلبيه نتيجه فحص النسيج لا تنفي التشخيص.

Introduction

Celiac disease (CD) has been defined traditionally as a small bowel disorder characterized by mucosal inflammation, villous atrophy, and crypt hyperplasia, which occur upon exposure to gluten and which demonstrate improvement with withdrawal of gluten from the diet [1, 2].

Testing for CD should be considered in the following groups of patients [2]:

1. Those with gastrointestinal symptoms including chronic diarrhea, malabsorption, weight loss, and abdominal distension.
2. Individuals without other explanations for signs and symptoms such as persistent elevation in serum aminotransferases, short stature, delayed puberty, iron-deficiency anemia, recurrent fetal loss, and infertility.
3. Symptomatic individuals at high risk for celiac disease including patients with type 1 diabetes mellitus or other autoimmune endocrinopathies, first- and second-degree relatives of individuals with celiac disease, patients with Turner, Down, or Williams syndromes.

As a general rule, testing should begin with serologic evaluation. The most sensitive and specific tests are IgA tissue transglutaminase antibody (IgA TTG) and IgA endomysial antibody, which have equivalent diagnostic accuracy. By contrast, antigliadin antibody tests are no longer used routinely because of their lower sensitivity and specificity.

IgA TTG was highly sensitive and specific for the diagnosis of CD in most reports. In an illustrative series, IgA TTG was present in 98 percent of patients with biopsy-proven CD compared to 5 percent of controls [3]. In another study that included 136 patients with CD and 207 controls, the sensitivity and specificity of IgA TTG was 95 and 94 percent, respectively [4].

ELISA tests for IgA TTG are now widely available and are easier to perform and less costly than the immunofluorescence assay used to detect IgA endomysial antibodies.[5-7] The diagnostic accuracy of IgA TTG immunoassays has been improved further by the use of human TTG in place of the non-human TTG preparations used in earlier immunoassay kits. [8]All diagnostic tests should be performed while the patient is on a gluten-containing diet.

Patients with a positive IgA TTG test should undergo a small bowel biopsy. Exceptions are those who have biopsy-proven dermatitis herpetiformis in whom the diagnosis can be established without a small bowel biopsy. Multiple biopsies should be obtained in the second and third portion of the duodenum. The exact minimal number is uncertain, although some experts believe that at least four should be obtained. [9, 10] The duodenal mucosa may appear atrophic with loss of folds, contain visible fissures, have a nodular appearance or the folds may be scalloped, but such findings are not universally present and may be seen with other disorders (Bacterial overgrowth, Crohn's disease, Cow's milk protein intolerance, Giardiasis, Lymphoma, Post gastroenteritis and Tropical sprue).[11]

Staining techniques and high resolution magnification endoscopy can help identify areas of villous atrophy for biopsy. [12-14]

The standard approach has been to obtain a small bowel biopsy for histopathologic examination. However, the very high specificity of the IgA TTG tests has led to debate as to whether a positive result in the appropriate clinical setting can be considered diagnostic and eliminate the need for small bowel biopsy.

This study was conducted to see if high TTG titer is enough to diagnose celiac disease without the need for small-bowel biopsy.

Patients and Methods

This case-control hospital based study was conducted in Karbala teaching hospital for children, Karbala, Iraq, on 116 patients with signs and symptoms suggestive of CD (chronic diarrhea, vomiting, anorexia, foul stool, irritability, abdominal distension, weight loss and short stature) over 2 years from January 2008 to January 2010.

All patients had undergone TTG testing (IgA&IgG class) in the hospital and small-bowel biopsy from second and third part of the duodenum at the Gastroenterology center in Al-Hussein hospital by an expert gastroenterologist. The biopsies were reviewed by a pathologist blinded to the patients, clinical history, TTG results, and previous biopsy reports. Villous atrophy with accompanying lymphocyte infiltration and cryptic hyperplasia were defined as characteristic histological findings of CD.

The commercial TTG kits used by our hospital laboratory during the examination period were AESKULISA tTg-A and AESKULISA tTg-G (AESKU. DIAGNOSTICS. MIKROFORUM RING 2.55234 WENDELSHEIM.GERMANY).

The manufacturer recommended the following TTG cutoff values:

Normal Range	Equivocal Results	Positive Results
< 12 U/ml	12-18 U/ml	>18 U/ml

Fifty healthy volunteers (control group), without family history of CD, were sent for TTG testing after taking oral consent from them or their families.

Ten patients excluded from the study because their TTG were ≤ 18 U/ml and their duodenal biopsies were negative.

Results

The mean age of patients in the study group was 5.2 (1-18) years and male / female ratio was 45 / 71 (0.7 / 1). Family history of celiac disease was seen in 26 / 106 (24.5%).Forty four patients(41.5%) were breast-fed up to 2 years of life.

The most common presenting symptoms and signs were : chronic diarrhea 100/106 (94%) , foul stool 96/106 (90.6%), abdominal distension 95/106 (89.6%), anorexia 82/106 (77.4%) ,failure to thrive 64/106 (60.4%),and short stature 60/106 (56.6%) as shown in (Table 1). TTG & Biopsy Results

Seventy two patients had IgA TTG values > 100 U/ml, of whom 70 exhibited positive biopsy results (97.2%),28 patients had IgA TTG values > 18-100 U/ml , of whom 22 exhibited positive biopsy results (78.6%), while only 6 patients with IgA TTG levels ≤ 18 U/ml had positive biopsies (Table 2).

Forty one patients had IgG TTG values > 18-100 U/ml, of whom 38 exhibited positive biopsy results (92.7%), 28 patients had IgG TTG values > 100 U/ml,of whom 26 exhibited positive biopsy results (92.9), while 37 patients had IgG TTG values ≤ 18 U/ml, of whom 34 exhibited positive biopsy results (91.9%) as shown in (Table 3).

Table 1. Presenting Symptoms and Signs in the Study Group

Symptoms & Signs	Number of Patients	%
Chronic diarrhea	100	94.3
Foul stool	96	90.6
Abdominal distension	95	89.6
Anorexia	82	77.4
Failure to thrive	64	60.4
Short stature	60	56.6
Vomiting	56	52.8
Irritability	36	34
Wasted muscles	36	34

Table 2. IgA TTG Levels According to Small Bowel Biopsy Results.

Biopsy	No. With IgA TTG Level			
	≤18 U/ml	>18-100 U/ml	> 100 U/ml	Total
Positive	6	22	70	98
Negative	0	6	2	8
Total	6	28	72	106

Table 3. IgG TTG Levels According to Small Bowel Biopsy Results

Biopsy	No. With IgG TTG Level			
	≤ 18 U/ml	> 18-100 U/ml	> 100 U/ml	Total
Positive	34	38	26	98
Negative	3	3	2	8
Total	37	41	28	106

Six patients had negative IgA TTG results (≤ 18 U/ml) but their biopsies were positive (sensitivity of IgA TTG= 94.3%).

Thirty four patients had negative IgG TTG results (≤ 18 U/ml) with positive biopsy results (sensitivity of IgG TTG 67.9%).

Eight patients had negative biopsy results while their IgA TTG values were > 18 U/ml (sensitivity of duodenal biopsy =92.6%).

Six patients with normal or equivocal IgA TTG results (≤ 18U/ml), had positive biopsies and positive IgG TTG results.

The IgA TTG results were normal or equivocal (≤ 18U/ml) in 48/50 of control group, 2 volunteers had their IgA TTG values 21 and 24U/ml respectively (specificity of IgA TTG = 96%).

Discussion

The majority of patients were 1-5 years 59/106 (55.7%), 33/106 (31.1%) were 6-10 years, while only 14 patients (13.2%) were above 10 years. In most European studies, the disease is diagnosed before the end of second year of life[15], while in our study the mean age of presentation was 5.2 years. This changing presentation of the disease may be due to longer periods of breast-feeding (44/106 of our patients were breast-fed up to 2 years of life),late introduction of gluten into infant diet, and failure of health care professionals to recognize the variable clinical manifestations of CD and to perform the appropriate tests to make the diagnosis. [16, 17]

Chronic diarrhea, foul stool, abdominal distension, and anorexia were the main presenting symptoms & signs (Table 1), while all patients with failure to thrive &

short stature had positive biopsies for celiac disease, as well as an IgA TTG level > 100 U/ml, this means mucosal damage will be great & extensive with these 2 presentations & the sensitivity & titer of IgA TTG will be higher with greater degree of mucosal damage.[18, 19]

It is currently recommended that confirmation of the diagnosis of CD requires an intestinal biopsy in all cases. A clinical diagnosis in children on the basis of gastrointestinal symptoms alone was incorrect in more than 50% of cases.[20, 21]

Eight symptomatic patients with +ve IgA TTG (>18U/ml), had –ve duodenal biopsy. Negative small-bowel biopsy could be due to patchy mucosal involvement with variable severity [22].In some cases a biopsy from one site has total villous atrophy whereas that from adjacent site was normal or showed only mild lymphocyte or plasma cell infiltration of the lamina properia[23]. It is recommended that multiple endoscopic biopsies be obtained from the more distal segment of the duodenum. Areas with a mucosal mosaic pattern or scalloping of the duodenal folds, when present, are preferred sites for obtaining a biopsy.[11]

From the above studies [11,22,23],we can conclude, why the sensitivity of duodenal biopsy in this study(92.6%)was not very high, moreover the specificity of small-bowel biopsy is not very high too, since the histological findings may be seen in other disorders, like bacterial overgrowth, cow's milk protein intolerance, giardiasis, post gastroenteritis….etc.[13]

Seventy two patients had high titer IgA TTG >100U/ml (mean=324 U/ml), of whom, only 2 had negative small-bowel biopsy (sensitivity of high titer IgA TTG 97.2%). All patients responded to gluten free diet and all their symptoms improved, 3-6 months later IgA TTG titer of most patients was <18U/ml, especially those with strict diet regime, these findings support our diagnosis and were in line with other studies.[24-28] The specificity of IgA TTG was 96% according to the manufacturer cutoff values while it was 100% if we increase the cutoff value to >100U/ml, this means the sensitivity of high cutoff value of IgA TTG 97.2% , specificity 100%, positive predictive value 100% and small-biopsy is not necessary if the patient symptoms improved on gluten free diet.

The IgG TTG test has been known as a research tool since 2000.It has not reached widespread clinical use but would be useful for patients with IgA deficiency , to screen them for celiac disease [29,30] (the estimated prevalence of selective IgA deficiency in celiac disease varies from 1-5%). [31-33]

IgG TTG test is used in our hospital in conjunction with IgA TTG test to screen for celiac disease without assessment of the IgA status of the patients.

Six patients had positive small-bowel biopsies, their IgA TTG values<18U/ml, and their IgG TTG values > 100U/ml (mean=221U/ml). All 6 patients improved symptomatically on gluten free diet.

There are three main possibilities in those with suggestive clinical features but –ve IgA TTG:

1. The patient may have selective IgA deficiency. In such cases, testing for IgG TTG should performed and it was +ve in all the 6 cases.
2. The serological tests could be false –ve (if the IgG TTG was also –ve), in such cases, it should be repeated or a small bowel biopsy should be obtained.
3. The patient may not have CD ,in such conditions other causes for the symptoms and villous atrophy should be considered.[34]

Conclusions

1. Symptomatic patients with IgA TTG or IgG TTG >100 U/ml can be treated as celiac disease without doing duodenal biopsy, the latter is considered if the patient exhibited no or poor clinical response to strict gluten free diet.

2. Negative duodenal biopsy doesn't exclude CD in a symptomatic patient.

Recommendation

The use of higher titer IgA TTG cutoff values than currently recommended will improve the specificity of the test and its positive predictive value.

Acknowledgments

I would like to express my gratitude to the gastro-entrologist dr. Hadi Abed Zaid,pathologists dr. Nazar Jabar & dr. Haider Jabur, dr. Hussein Ne'ma,and biologists Hanan Mahdi & Essa Al-Hashemi, dr.Saad Al-Gabban and Zahraa Al-Musawi for their unlimited help and support..

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