Correlation between the Level of Anti-Tissue Transglutinmase IgA and Marsh Classification in Celiac Disease

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Abstract

Aim of study: evaluate the level Anti-tissue transglutinmase Anti body titer with the histopathological marsh classification in celiac disease patients

Patients and materials: Fifty patients included in this study through the period between 2009-2010 All patients on gluen diet at the time of serum collection and histiopathological biopsy. all the patients included in this study above the age of 18 years . 44 patients present with G.I.T. symptoms 6 patients presented with non gastrointestinal symptoms oral endoscopic biopsy was taken from duodenum, The biopsy put in formalin fixation(10%), paraffin block and the slides was stained by (H&E), The Histopathological assessment for a celiac disease according to marsh classification, serum was collected and antitissue transglutinmase IgA Antibodies measured by ELIZA.

Results: the histiopathological findings of second part duodenal biopsy as a following 8(16%) cases with Marsh I(intraepithelial lymphocytes infiltration), 10 (20%) cases with Marsh II (intraepithelial lymphocytes infiltration +crypt hyperplasia),16 (32%) cases Marsh IIIA (intraepithelial lymphocytes infiltration +crypt hyperplasia+ partial villous atrophy), 10 (20%) cases with Marsh IIIB (intraepithelial lymphocyte+crypt hyperplasia+ subtotal villous atrophy) 6 (12%) cases with Marsh IIIC(intraepithelial lymphocytes +crypt hyperplasia +total villous atrophy).

Conclusion: In the present study the serum level of anti-tissue transglutinmase IgA anti body was higher in celiac with severe entropathy (Marsh IIIb-c) than in those with slight entropathy (Marsh I-IIIa), so the serological tests with high level>100 IU/ml reveal in celiac disease while low serological test need histophalogical assessment to avoid underestimate the real prevalence of celiac disease.

الخلاصة

الأهداف: تقييم العلاقة بين مستوى إنزيم ترانز كلتوميز النسيجي مع تصنيف مارش لمرضى حساسية الحنطة.

الطريقة :اجريت الدراسة على ٥٠ مريض خلال الفترة الممتدة من ٢٠٠٩-٢٠١٠ جميع المرضى مستمرين على غذاء يحتوي على الحنطة و ٤٤ مريض يعانون من أعراض الجهاز الهضمي مثل الإسهال المزمن ، الآلام البطن ، عسر الهضم والإمساك . ٦ مرضى يعانون من أعراض مرضية لا علاقة لها بالجهاز الهضمي مثل قصر القامة ، فقدان الوزن ، فقر الدم والإعراض العصبية. جميع المرضى فوق سن ١٨ سنة اجري لهم ناظور المعدة والأمعاء الدقيقة وتم اخذ خزعة نسيجية من الجزء الأول من الأمعاء الدقيقة ووضع العينات في ١٠% فورمالين ، وا ٍجراء المعاملة بالكحول والزايلين ووضع العينات في قوالب الشمع ثم صنع السلايدات وصبغها بصبغة هيماتوكسين والايوسين ، اجري الفحص النسيجي على العينات من اجل تشخيص وتقييم مرضى حساسية الحنطة حسب تصنيف مارش ثم تم جمع مصل الدم من المرضى واجري فحص مستوى الأجسام المضادة لإنزيم انسجة ترانز كلتوميز

النتائج :أظهرت الدراسة ٨ حالات مارش I ، ١٠ حالات مارش II ، ١٦ حالة مارش IIIA ، ١٠ حالات مارش IIIB و٦ حالات مارش IIIC .وأظهرت كذلك الحالات الموجبة لفحص الانزيم هي حالة واحدة وضعيفة مارش I ، ٨ حالات مارش II ، ١٣ حالة مارش IIIA ، 61.5% حالة من هذه الحالات موجبة متوسطة ، وجميع حالات مارش IIIB وIIIC موجبة قوية (iu 100>) وبنسبة ٢٥% و ١٠٠% على التوالي

الاستنتاج: في هذة الدراسة مستوى مصل الدم الجسام المضادة لإنزيم تانزكوتمنيزالنسيجي كان عاليا في مرضى حساسية الحنطة بدرجةIIIcمن المرضى بدرجةIIIa-b و مستوى الانزيم العالي أكثر من ١٠٠ يشير إلى تشخيص حساسية الحنطة بينما مستوى الإنزيم الواطئ ٢٥-٥٠ يحتاج إلى الفحص النسيجي.

Introduction

Celiac disease is an immune-mediated inflammation of the small intestine caused by sensitivity to dietary gluten and related proteins in genetically sensitive individuals.[1] The disorder is common, occurring in 0.5 to 1 percent of the general population in most countries[2]. gastrointestinal symptoms in adults are paradoxically, the disease may cause either constipation or diarrhea. When diarrhea is present, the stools are often bulky and foul-smelling, and may float because of steatorrhea. Flatulence and abdominal distension (caused by colonic bacterial digestion of malabsorbed nutrients) are common. These symptoms may be accompanied by the consequences of malabsorption, such as growth failure, weight loss, severe anemia, neurologic disorders from deficiencies of B vitamins, and osteopenia from deficiency of vitamin D and calcium. [3,4] .Celiac disease is frequently associated with dermatitis herpetiformis, Down syndrome, selective IgA deficiency, and other conditions which have autoimmune features such as type 1 diabetes mellitus, thyroid disease, and liver disease.[5,6,7]

The celiac lesion in the proximal small intestine was first described in 1954. The primary findings were mucosal inflammation, crypt hyperplasia, and villous atrophy.[8] The genetic basis of the disease is shown by the frequent intrafamilial occurrence and the remarkably close association with the HLA-DQ2 and/or DQ8 gene locus.[9] While the presence of either the HLA DQ2 or DQ8 genotype is essential to confer disease.

Testing for celiac disease should be considered in the following groups of patients

Those with gastrointestinal symptoms including chronic or recurrent diarrhea, malabsorption, weight loss, and abdominal distension or bloating,[10] Individuals without other explanations for signs and symptoms such as iron deficiency anemia, folate or vitamin B12 deficiency, persistent elevation in serum aminotransferases, short stature, delayed puberty, recurrent fetal loss, low birthweight infants, and reduced fertility persistent apthous stomatitis, dental enamel hypoplasia, idiopathic peripheral neuropathy, nonhereditary cerebellar ataxia, or recurrent migraine headaches.[11] Symptomatic individuals at high risk for celiac disease including patients with type 1 diabetes mellitus or other autoimmune disorders, first- and second-degree relatives of individuals with celiac disease, patients with Turner, Down, or Williams syndromes[12].

Diagnostic Approach:

All testing should be performed while patients are on a gluten-rich diet. No single test can confidently establish the diagnosis of celiac disease in every individual. As a result, the most important initial step in diagnosis is recognition of the many clinical features that can be associated with the disease.[13]

Serologic evaluation — As a general rule, testing should begin with serologic evaluation. As will be discussed below, the most sensitive and specific tests are IgA anti tissue transglutaminase and IgA endomysial antibody, which have equivalent diagnostic accuracy[14], TTG assays used guinea pig protein subsequent cloning of the humen TTG protein the sensitivity of TTG in both children and adult ranges from 92-100% the specifity of TTG in both range 91-100%, ther is good evidence that EMA and TTG are highly sensitive and specific tests for identifying individuals with celiac disease [15].

The diagnosis of celiac disease typically requires histologic changes on small intestinal biopsy in a symptomatic individual and Complete symptom resolution on a gluten free diet, the second , third and fourth parts of duodenum and the proximal jejunum has demonstrated suitable for diagnosing celiac disease [16], there is good evidence that the mucosal changes in celiac disease may be patchy in nature and vary in severity. Areas with a mucosal mosaic pattern or scalloping of the duodenal folds when present, are preferred sites for obtaining a biopsy [17]. The characteristic changes in celiac disease include an increased number of intraepithelial lymphocytes(>20 lymphocytes/100 entrocytes), an intraepithelial lymphocyte mitotic index greater than 0.2%, loss of nuclear polarity with pseudostritification of the epithelial cells, a decrease, in the number of goblet cells[18]. Structural changes include elongation of the crypts (increased crypt length), partial to total villous atrophy and a decreased villous: crypt ratio. Lamina propria changes include infiltration of plasma cells, lymphocytes, mast cells and eosinophils[18,19].

Histological grading systems used include the conventional system and that introduced by Marsh. Which is classified the histologic changes of celiac disease as Type 0 or preinfilterative stage(normal), Type 1 or infilterative lesion (increased intraepithelial lymphocytes), Type 2 or hyperplastic lesion (type1+hyperplastic crypts), Type 3 or destructive lesion (Type 2+ variable degree of villous atrophy) and Type 4 or hypoplastic lesion(total villoua atrophy with crypt hypoplasia. Type 3 has been modified to include Type3a(partial villous atrophy) Type 3b (subtotal villous atrophy) and Type 3c (total villous atrophy)

Patients and Materials

Were Fifty patients included in this study through the period between 2009-2010

All patients on gluen diet at the time of serum collection and Histiopathological biopsy.

44 patients present with G.I.T. symptoms included diarrhea, abdominal pain, weight loss and short stature, all the patients included in this study above the age of 18 years.

6 patients presented with non gastrointestinal symptoms including anemia and neurological symptoms. Oral endoscopic biopsy was taken from duodenum,

The biopsy put in formalin fixation (10%), paraffin block and the slides was stained by (H&E).

The Histopathological assessment for a celiac disease according to marsh classification system:

Marsh I: intraepithelial lymphocytes (> 20lymphocytes/100 entrocytes)

Marsh II: intraepithelial lymphocytes+ crypt hyperplasia

Marsh IIIA: partial villous atrophy

Marsh IIIB: Subtotal villous atrophy

Marsh IIIC: Total villous atrophy

Serum was collected and antitissue transglutinmase IgA Antibodies measured by ELIZA and the result assessed as following:

<12 IU/ml	negative
12-18 IU/ml	Equivocal
>18 IU/ml	positive

Results

The table (1) shows the histiopathological findings of second part duodenal biopsy as a following 8 (16%) cases with Marsh I (intraepithelial lymphocytes infiltration), 10 (20%) cases with Marsh II (intraepithelial lymphocytes infiltration +crypt hyperplasia),16

(32%) cases Marsh IIIA (intraepithelial lymphocytes infiltration +crypt hyperplasia+ partial villous atrophy), 10 (20%) cases with Marsh IIIB (intraepithelial lymphocyte+crypt hyperplasia+ subtotal villous atrophy) and 6 (12%) cases with Marsh IIIC(intraepithelial lymphocytes+crypt hyperplasia+total villous atrophy).

The Table (2) illustrate the Antitissue transglutinmase Ab titer level in patients were 38 (76%) cases are positive or equivocal > 12Iu/ml while 12 (24%) cases are negative < 12 IU/ml

The table (3) explain the level of antitissue transglutinmase Ab titer level in correlation with Marsh classification of histopathology : In marsh I only 1 case mild positive(25-50Iu/ml) , In marsh II 8 cases mild positive, In marsh IIIA 5 cases mild positive while 8 cases moderately positive (50-100IU/ml),Marsh IIIB 2 cases mildly positive , 6 cases moderately positive and 2 cases strongly positive(>100 IU/ml), In marsh III all 6cases strongly positive for TTGA.

Table 1 Histopathological Marsh findings

Histopathology of second doudenum	**No.**
Marsh I	8 (16%)
Marsh II	10 (20%)
Marsh IIIA	16 (32%)
Marsh IIIB	10 (20%)
Marsh IIIC	6 (12%)
Total	50 (100%)

Table 2 Antitissue Transglutinmase Ab titer level

TTGA Level	No. of patient
<12 (negative)	12 (24%)
>18(positive	38 (76%)

Table 3 Histopathological Marsh types with TTGA Ab level

Histopathological	**TTGA level (Equivocal) 12-18**	**TTGA level (Mild) 25-50**	**TTGA Level (Moderate) 50-100**	**TTGA level (high) >100**	**TTGA Negative <12**
Marsh I	5	1	0	0	2
Marsh II	1	8	0	0	1
Marsh IIIA	1	10	9	0	1
Marsh IIIB	0	1	4	2	0
Marsh IIIC	0	0	0	4	0
Total	7	20	13	6	4

Table 4 Clinical presentation of patients

Clinical presentation	No. of patient
Gastrointestinal tract	44 (88%)
Non Gastrointestinal tract	6 (12%)
Total	50 (100%)

Discussion

The mucosal changes in celiac disease may be patchy in nature and vary in severity because the histologic changes in celiac disease may be patchy , it is recommended that multiple biopsy specimens be obtained from the second or more distal part of the duodenum. There is good evidence that villous atrophy is a characteristic histopathological features of celiac disease[19].

The presence of infiltrative lymphocytes with crypt hyperplasia Marsh 2 on intestinal biopsy is compatible with celiac disease. The diagnosis of celiac disease is considered definitive when there is completely symptom resolution after treatment with gluten free diet in previously symptomatic individual with characterstic histological changes on small intestinal biopsy[20].

In the present study the Marsh I represent 16% of cases and this is higher than Tursi et al[21] and donaldsons etal[22] .(10.9%, 12%)

In our study the Marsh II 20% of cases which is similar to tursi etal and Donaldson etal 20.1%, 22%.

In our result the Marsh IIIa is 23% cases and this is different from Tursi etal 22.6% and slightly higher than Donaldson etal 28%

We found 20% of cases with Marsh IIIb which is less than Tursi etal 26% and similar to Donaldson etal 21%.

The Marsh IIIC 12% of cases is less than Tursi and Donaldson etal 20.1% and 17%.

The level of Antitisssue transglutinmase IgA Ab titer is strongly positive >100 IU/ml in Marsh IIIB&C, this is similar to Donaldson and tursi et al

While in the present study the Antitissue transglutinmase Ab positive in 2% of MarshI and this is less than tursi etal 7.6%.

In our study The marsh II positive Antitissue transglutinmase IgA Ab case about 70% and this is similar to tursi and Donaldson 68.5%, 71% respectively.

We found the Antitissue transglutinmase IgA Ab positive in MarshIIIA and MarshIIIB 87.5%, 90% which is slightly higher than Tursi and Donaldson etal 84%,88% respectively.

Conclusion and Recommendation

in present study the serum of anti-tissue transglutinmase IgA anti body was higher in celiac with severe entropathy (Marsh IIIb-c) than in those with slight entropathy (Marsh I-IIIa), so the serelogical tests with high level>100 IU/ml reveal to celiac disease while low serological test need histophalogical assessment to avoid underestimate the real prevalence of celiac disease.

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