The correlation of modified Marsh grading system in relation to clinico pathological and serological assessment in early diagnosis of celiac disease

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Abstract

Background: Celiac disease is an autoimmune disorder triggered by the ingestion of wheat gluten and related proteins of rye and barley in genetically susceptible individuals. Its diagnosis is based on clinical manifestations, endoscopic finding serological markers and histopathology. Aims: to correlate the degree of mucosal damage according to modified Marsh grading system histopathological with clinical finding, endoscopy and serology. Methods: in this cross sectional study, one hundred patients included, for each one upper GIT endoscopy, microscopic examination of biopsy specimens from duodenum, serum anti tissue transglutaminase antibodies level were analyzed. Results: 83% of patient have typical clinical feature of celiac disease but with accuracy (54.7%). The upper intestinal endoscopy reveal (53 %) of cases had features of celiac disease, with accuracy of (84.1 %) more than clinical finding in the diagnosis. A significant correlation (p value < 0.001) obtained between serum level of anti tTG antibody and histopathology with sensitivity of (95.7%) and specificity of (91.8%). In addition there is positive linear correlation between these two parameters (r=+0.942). Conclusion: Clinical presentations lack specificity. Endoscopy has relatively good sensitivity and specificity. Serum level of anti-tTG antibody have high sensitivity and specificity therefore, it is reliable mean for prediction and diagnosis of celiac disease.

Keywords: Celiac disease, Modified marsh grading system, anti-tissue transglutaminase antibody

Introduction

Celiac disease or gluten-sensitive enteropathy or non-tropicalsprue or endemic sprue, is an autoimmune disorder that triggered by ingestion of wheat gluten and related proteins in genetically susceptible peoples [1], these proteins found in grain species like wheat, barley and rye, which may cause damage in the small intestine mucosa, result in disordered absorption [2]. It characterized by chronic inflammatory process that gradually leading to the development of mucosal villous atrophy. Gluten free diet leads to clinical and histological improvement, while its re-consumption results in recurrence of the disease [3]. The prevalence of CD is estimated to be around 1% in the general population [4]. Non-invasive methods are increasingly important in the diagnoses of CD, New options for diagnosis of celiac disease have been discovered in addition to the well-known biochemical, hematological and other methods due to progress in clinical genetics and immunology, at the same time, the detection of circulating auto-antibodies is becoming more frequent in the clinical practice [5]. Unrivaled feature of celiac disease that is important in diagnosis and screening was production of IgA specific antibody for tissue transglutaminase (tTG) enzyme in circulation [6], despite it is very specific serologic tests, duodenal biopsy still the gold standard for diagnosis of CD [1]. However, the diagnosis of celiac
disease is based on clinical characteristics, serology, and histology of the small intestine mucosa [7]. 90-95% of patient with CD had HLA DQ2 and the remaining 5-10% had HLA-DQ8, these haplotypes are carried by about 30% of the unaffected population also [8]. It is assumed that HLA-DQ2/DQ8 demonstrate <50% of the genetic explanation of its ‘etiology’ [9], so there must be other genetic factors important for playing a role in development of CD. Serology (antigliadin antibody, anti-endomysial antibody and tissue transglutaminase antibody) has altered algorithm of diagnosis [10]. At first, only AGA was used, but nowadays, this antibody has been greatly exchanged by EMA and tTGA, many patients have IgA antibody against whole gladding, gluten, but this antibodies have specificity for celiac disease much lower than the anti-(tTG) IgAELISA assay [11], tTG-IgA in adults and most children had a sensitivity and specificity more than 90% and 95%, respectively [12]. EMA-IgA commonly associated with tTG. But it is more expensive and identified manually; therefore it is not the first line marker [12]. The tTG-IgA test is an enzyme-linked immunosorbent assay (ELISA) test and is the preferable screening method with high sensitivity, yielding just few false negative and false positive results. [13] The tTG-IgG test is only useful in those people who have IgA deficiency, they form 1/400 of the general population or 2% to 3% of people with CD [14]. Although high titers in the serology are indicative of CD, they alone do not provide a definitive diagnosis and a biopsy from the small intestine is required for a definitive diagnosis [15]. Microscopic enteritis is associated with symptoms of micronutrient deficiency, characterized by subtle mucosal abnormalities (Marsh 0-II) without prominent inflammation, villous atrophy, erosions or ulcerations as seen by light microscopic examination [16].

Histopathology
The currently used grading system for celiac disease in small bowel biopsies is modification of Marsh (Marsh Oberhuber) criteria which include [17]:

**Grade 0**: In which the intestinal lining is normal, so celiac disease is unlikely.

**Grade 1**: In which lymphocytes have started to move into the surface of the intestinal lining [increase intraepithelial lymphocytes] only (> 25 lymphocytes/100 epithelial cell).

**Grade 2**: In which even more lymphocytes appear in the intestinal lining and the lining itself starts to look abnormal, with larger-than-normal depressions between the intestinal villi [crypt proliferation or hyperplasia].

**Grade 3**: In which there is grade 2 changes (increase intraepithelial lymphocytes, plus crypt hyperplasia). In addition, in grade 3 the villi themselves are starting to shrink and flatten [villous atrophy], there are 3 subsets of grade 3:
- Partial(mild) villous atrophy (grade 3a)
- Subtotal (moderate) villous atrophy (grade 3b)
- total (severe) villous atrophy (grade 3c)

**Grade 4**: In which the villi are totally atrophied (completely flattened), and the depressions between them have shrunk, too, atrophic hypo plastic lesion; flat mucosa with normal crypt height and no significant inflammation with normal intraepithelial lymphocytes counts. A proportion of people with normal mucosa (Marsh 0) and positive CD serology may in fact have microscopic enteritis, research showing that individuals with endoscopic ally normal mucosa but positive CD serology often progress to Marsh I-III with symptoms consistent with CD [18].

Serological tests
They are routinely used as potent screening tool for CD and it has been established that they have superior value in diagnosing celiac disease compared to milder enteropathy, in developing countries many persons with even classical CD are more likely to stay undiagnosed due to limited resources with poor access to intestinal biopsy and serology and this may explain why some patients presenting with overt malabsorption. It was found that celiac disease progress
gradually from small intestinal mucosal inflammation to crypt hyperplasia while only a few cases may develop overt villous atrophy [19].

The serologic tests detect three antibodies common in CD:
1-Tissue transglutaminase (tTG) antibodies
2-Endomysial antibodies (EMA)
3-Deamidated gliadin peptide (DGP) antibodies

The tTG-IgA test has sensitivity in some studies of 93%, yielding few false negative results and specificity of 98% [20]. Regarding EMA is measured by indirect immunofluorescent assay, but it is costly and time-consuming process more than ELISA testing. In addition, the EMA test is qualitative, so the results are more subjective than those for tTG [21]. A new generation of tests are those using deamidated gliadin peptide (DGP) antibodies with sensitivity and specificity that is substantially better than the older gliding tests however there is insufficient evidence to support the use of DGP over tTG or EMA tests and tTG test is less expensive than the DGP test and provides a better diagnostic performance [22].

Endoscopy

CD can present with nonspecific upper gastrointestinal symptoms. So patients may undergo esophagogastroduodenoscopy as their initial investigation. Endoscopically the markers of villous atrophy are well described and might have limited sensitivity for patients with mild enteropathy and therefore duodenal biopsies should be performed if there is high suspicion of CD irrespective of endoscopic appearance [23], histopathology revealing villous atrophy in most of who had celiac disease. Among these, most having endoscopic finding of mucosal mosaic pattern in the duodenal lining, many having scalloped duodenal folds, few having visible vasculature and reduction of duodenal folds. Except for the mosaic pattern, the frequency of endoscopic abnormality increased with age; while reduction of duodenal folds rarely seen in children less than 5 years old [24]. (CD) diagnostic testing can be summarized in the following algorithm [25]:

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DGP, deamidated gliadin peptide; HLA, human leukocyte antigen; Ig, immunoglobulin; TTGA, tissue transglutaminase antibody
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Method and materials

This prospective cross sectional study had been carried out from December 2018 through December 2019 in collaboration between gastroenterology center (with its serology lab unit) and the histopathological lab unit at Al Sader teaching hospital in al Najaf province. The clinical data were depended on clinical presentation and endoscopic finding of the upper gastrointestinal tract done to all 100 patients of the study and the biopsy specimens taken endoscopically from duodenum) were submitted for histopathological examination and microscopic finding of celiac disease were assessed and graded according to modified Marsh grading systems into four grades (score 0, 1, 2 and 3). All included patients have underwent endoscopic examination, in addition to serology test (anti tTG Ab) and biopsy for histopathology examination. All biopsy specimens
were examined microscopically for the villous-crypt ratio, the mucosa of the intestine, and in small intestinal biopsies (with cutoff of 25 lymphocytes/100 duodenal epithelial cells) and classified into 4 grades (from 0-3). Those patients with histological features suggestive of celiac disease were of Marsh grade 1 or more, while those with Marsh 0 were defined as of normal mucosa or mild nonspecific inflammation of lamina propria. Those patients were selected based on their upper GIT presentation of malabsorption. We assess the diagnosis of celiac disease based on histopathological examination of small intestinal biopsy according to modified Marsh grading system, clinical assessment (clinical features/endoscopic finding) and serological results (anti tTG Ab level) in double blinded manner (All workers including the second histopathology’s didn’t know anything about this study to avoid bias).

**Serological study**

The serology samples were taken for anti tissue transglutaminase antibodies (anti tTG Ab) assessed using AESKULISA kit and the results based on the following interpretation:

<table>
<thead>
<tr>
<th>Normal-range</th>
<th>Equivocal-range</th>
<th>Positive-range</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12 U/ml</td>
<td>12-18 U/ml</td>
<td>&gt; 18 U/ml</td>
</tr>
</tbody>
</table>

**Handling and histological evaluation of biopsy**

The biopsies were taken from the same sites in all of the studied patients then the biopsy specimen put in gel foam and filter paper to preserve the orientation of the mucosal villi and prevent malorientation, then sections made by perpendicular cut on mucosal surface so all layers of specimen and especially the mucosa can be evaluated properly. After processing embedding and sectioning, stained by Hematoxylin and eosin stain and examined microscopically by two pathologists. We assess the number of intraepithelial lymphocytes by counting those that are more close to the tip of villi and can be differentiated from the nuclei of enterocytes by being round and of dense chromatin while the nuclei of enterocytes are elongated or oval with more open chromatin as appear on H&E sections, however sometimes tangential sections can give them rounded appearance mimic that of lymphocytes. To consider the increase of intraepithelial lymphocytes, their count should be at least 25 in 100 enterocytes. We also assess the crypt hyperplasia and the villous length by assessing 3-4 consecutive villi by measuring villous to crypt ratio which should be more than 3:1.

**Statistical analyses**

It is done by SPSS version 24.IBM 2015. Categorical variables were presented as frequency and percentage. P-value of < 0.05 was considered as significant.

**Results**

The upper GI endoscopy with concentration on duodenal mucosa reveal in 53 patients (53% ) features suggestive of celiac disease like scalloping, mosaic pattern, reduction of duodenal folds and visibility of submucosal blood vessels, while there is no remarkable features in 47 other cases (47%).

![Figure (4-1): Distribution of endoscopic findings among the studied group](http://doi.org/10.36295/ASRO.2020.231436)
As this table shows, there is poor accuracy of clinical finding in predicting celiac disease.

Table (4-2): Correlation between Marsh grading and clinical findings

<table>
<thead>
<tr>
<th>Marsh grading</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marsh 0</td>
<td>51</td>
<td>51.0</td>
</tr>
<tr>
<td>Marsh 1</td>
<td>15</td>
<td>15.0</td>
</tr>
<tr>
<td>Marsh 2</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>Marsh 3</td>
<td>29</td>
<td>29.0</td>
</tr>
<tr>
<td>Marsh 3a</td>
<td>8</td>
<td>8.0</td>
</tr>
<tr>
<td>Marsh 3b</td>
<td>11</td>
<td>11.0</td>
</tr>
<tr>
<td>Marsh 3c</td>
<td>10</td>
<td>10.0</td>
</tr>
</tbody>
</table>

This correlation shows that endoscopic features are more accurate than clinical features in diagnosis of celiac disease.

Table (4-3): Correlation between Marsh grading and endoscopic findings

<table>
<thead>
<tr>
<th>Marsh grading</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoscopic finding</td>
<td>Positive (n = 49)</td>
</tr>
<tr>
<td>Suggestive (Typical)</td>
<td>43</td>
</tr>
<tr>
<td>Not suggestive</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
</tr>
</tbody>
</table>

Validity tests of clinical findings
| Sensitivity | 87.8% |
| Specificity | 80.4% |
| Accuracy | 84.1% |

Table (4-4): Distribution of serum level of anti tTGAb among the studied groups

<table>
<thead>
<tr>
<th>Serum level of anti tTGAb (U/ml)</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 12</td>
<td>50</td>
</tr>
<tr>
<td>12 - 18</td>
<td>0</td>
</tr>
<tr>
<td>&gt;18</td>
<td>50</td>
</tr>
<tr>
<td>SD = 90.96</td>
<td></td>
</tr>
<tr>
<td>N = 100</td>
<td></td>
</tr>
</tbody>
</table>

A significant correlation \( (p \text{ value } <0.001) \) was obtained between serum level of anti tTG antibody and histological features graded according to Marsh system, although there is no much difference in levels obtained between Marsh 1 and 2, and between Marsh 3a and 3b, however there is marked increase in level obtained in Marsh 3c.

Table (4-5): Comparison of mean anti tTG level across Marsh grading

<table>
<thead>
<tr>
<th>Marsh grading</th>
<th>No. of patients</th>
<th>Range of Serum level of anti tTGAb (U/ml)</th>
<th>Total no. of patients</th>
<th>Mean level of total</th>
<th>Serum level of anti tTGAb (U/ml)</th>
<th>SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marsh 0</td>
<td>48</td>
<td>0.1 – 11.3</td>
<td>51</td>
<td>3.47</td>
<td>3.47</td>
<td>5.53</td>
</tr>
<tr>
<td>Marsh 1</td>
<td>15</td>
<td>18.4 – 70</td>
<td>15</td>
<td>31.74</td>
<td>31.74</td>
<td>15.15</td>
</tr>
<tr>
<td>Marsh 2</td>
<td>4</td>
<td>43.4 – 60.86</td>
<td>5</td>
<td>43.79</td>
<td>43.79</td>
<td>23.42</td>
</tr>
<tr>
<td>Marsh 3a</td>
<td>8</td>
<td>106.9 – 190.8</td>
<td>8</td>
<td>136.27</td>
<td>136.27</td>
<td>64.09</td>
</tr>
<tr>
<td>Marsh 3b</td>
<td>10</td>
<td>23.75 – 200.4</td>
<td>11</td>
<td>137.90</td>
<td>137.90</td>
<td>31.39</td>
</tr>
<tr>
<td>Marsh 3c</td>
<td>10</td>
<td>211 - 300</td>
<td>10</td>
<td>275.85</td>
<td>275.85</td>
<td>33.61</td>
</tr>
</tbody>
</table>

P. value < 0.001
*SD: standard deviation of the mean.

Figure (4-2): Comparison of mean anti tTG level across Marsh grading

Sensitivity = 95.7%, Specificity = 91.8%, Accuracy = 93.8%

Figure (4-3): ROC analysis for the validity of anti tTG level in prediction of Marsh grade (AUC; area under the curve)

Figure (4-4): Endoscopic picture of scalloping pattern of the duodenal mucosa in CD

Figure (4-5): Endoscopic picture of mosaic pattern of duodenal mucosa in CD
Figure (4-6): Duodenal biopsy show in intact duodenal mucosa with normal intraepithelial lymphocytes count and villus/crypt ratio of 3/1 or more and was given Marsh 0 (x 10 objective H&E stain)

Figure (4-7): Duodenal biopsy showing increase intraepithelial lymphocytes, but the villi have normal length, and were given Marsh 1 (x 40 objectives H&E stain)

Figure (4-8): A. Duodenal biopsy showing marked villous atrophy with crypt hyperplasia and intraepithelial lymphocytosis, and was given Marsh 3c (x10 objectives H&E stain)

Figure (4-8): B. Same case above with higher magnification (x 40 objectives)

Figure (4-9): Duodenal biopsy showing moderate villous shortening with increase intraepithelial lymphocytes and crypt hyperplasia, and was given Marsh 3b (x 10 objectives H&E stain)
Figure (4-10): Duodenal biopsy showing complete absence of villi with marked intraepithelial lymphocytosis and was given Marsh 3c (x 10 objectives H&E stain)

Discussion

The diagnosis of celiac disease based on clinical presentation, endoscopic finding serology, and histology of small intestine mucosa [7]. According to this study, the association between the clinical features of patients and the histological changes of duodenal mucosa that suggest celiac disease although was with relatively good sensitivity (about 87.8%), it has poor specificity (about 21.6%) so hindering the clinical features from being reliably dependent on this correlation. However, other studies give poor sensitivity like in Kassem et al. which show sensitivity 27.8% [26]. According to this study most of cases with histological morphology compatible with celiac disease also found to have upper GI endoscopy finding suggestive of the disease (about 87.8% of patients with positive morphology), with sensitivity and specificity of 87.8% and 80.4% respectively, this finding has also documented by other studies like Shivani et al. in India [27] while few studies are not agreed with our results like Kassem et al study [26] also E. Akin and O. Ersoy in Ankara, Turkey [28]. By histological examination of submitted endoscopic biopsy specimens, we found in our study only about half of the specimens (49%) to have morphologic features consistent with celiac disease with variable grades (predominantly grade 3) according to Marsh grading system, with serum level of anti- tTG antibody of IgA type were correlated with histological features for each patient. In those with Marsh 0, the mean level of anti- tTG antibody was found to be (3.47 U/ml) i.e in negative range according to AESKULISA method, while significant increase in the mean of antibody level was noticed as the Marsh grade increasing, however, there is little difference but still increasing between Marsh 3a (mean level 136.27 U/ml) and Marsh 3b (mean level 137.90 U/ml), and this study showed that there is very strong positive linear correlation between serum level of anti- tTG antibody and severity of mucosal damage graded according to Marsh system, \( r = +0.942 \), which is statistically significant (p value < 0.001), similar results obtained by Peter Makovicky et al [29] and also by some other studies that use Corazza system which has simplified but similar morphologic divisions as Marsh system like the one done by Jan et al [30]. The validity of anti- tTG antibody serological level across Marsh histology in this study was of sensitivity of (95.7%) and specificity of (91.8%) this finding is approximately similar to that obtained by Lewis NR, Scott BB, Leffler, D.A. and Schuppan, D [31]. Some studies show higher sensitivity specificity [32-33], like Saeeda Almarzooqi, Ronald H. Houston, and Vinay Prasad [34], while according to other researches there is relatively poor sensitivity but higher specificity, as in the study by Kassem et al. Paradoxically, this study show two cases in which there is mucosal injury consistent with celiac disease (Marsh 2, Marsh 3b) along with positive relevant clinical features and endoscopic finding but have negative anti- tTG antibody serum level which diagnosed as celiac disease, one of the explanations is IgA deficiency state. This paradox was also obtained by others, as reported by Virchows Archiv, Villanacci V, Saeeda Almarzooqi, Ronald H. Houston, and Vinay Prasad [34], also obtained by Peter Makovicky et al. Other finding in this study is the presence of 3 cases with unremarkable histological morphology but have positive serology (anti tTG antibody) this is against our previous results, this also obtained by others [21], like Shivani Kalhan et al. Such individuals are at increased risk of future celiac disease, however there is possibility that some
people with positive serology and normal mucosa do not suffer from a raised risk for future celiac disease and this positive serology can sometimes be transient.

**Conclusions**

According to the results of this study we can conclude the following:

1- The clinical features lack specificity in prediction of CD and therefore can't reliably depend upon.

2- Upper gastrointestinal endoscopy have relatively good sensitivity and specificity in identifying features of CD and is an important step for suggestion and obtaining biopsy to reach the definite diagnosis.

3- Serum level of anti-tTG IgA antibody have high sensitivity and specificity, therefore, it is reliable mean for prediction and definitive diagnosis of celiac disease.

**Recommendations**

Any patient with suspected celiac disease should have serological assessment with most reliable and cost effective serological marker which is anti-tTG antibody and to have endoscopy and biopsy to confirm final diagnosis. In cases of IgA deficiency the patients may have negative serum anti-t TG IgA antibody level in spite of positive Marsh histological features so require measurement of total serum IgA level and make other measurement like anti-tTG IgG instead of IgA and anti-tTG IgG/DGP IgG antibody combination. This condition may be also obtained in cases of latent celiac disease in which the patient may or may not be symptomatic with positive serology but negative histological features.

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