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Modified Marsh Classification of the Duodenal Biopsies of a Large Database Covering 10 Years

On Yılı Kapsayan Duedonal Biyopsi Kayıtlarının Modifiye Marsh Klasifikasyonu

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ABSTRACT

Purpose: Celiac is an autoimmune disease caused by of gluten proteins which can be found in multi-grain food like wheat, barley and oat. The disease affects more than 1% of population and characterized by intestinal inflammation. In celiac disease, mucosal damage is a dynamic process. It is shown that it has autoimmune components. It is also T-Cell mediated and can be categorised as a chronic inflammatory disease. The purpose of this study is to make modified Marsh classification of the duodenal biopsies that came to our department in the 10 years. The study deals with reassessment of all events and uncovering the low graded events that were not diagnosed.

Material and Methods: 467 biopsies (diagnosed between 2001 and 2011 at the Cukurova University, Faculty of Medicine, Department of Pathology) were taken and analyzed by two pathologists. Each sample was reevaluated without taking the previous reports into consideration and scored by using modified Marsh classification.

Results: According to Modified Marsh Classification total of 48 cases were diagnosed as Type 1. Total of 6 cases according to Modified Marsh Classification was diagnosed as Type 2. Total of 11 cases according to Modified Marsh Classification was diagnosed as Type 3a. Total of 5 cases, according to Modified Marsh Classification, was diagnosed as Type 3b. Total of 6 cases according to Modified Marsh Classification was diagnosed as Type 3c.

Conclusion: As a result of this study, it has been found that Modified Marsh Classification is a very important standardization tool for detection of suspicious duodenal biopsies and for early case examinations.

Key Words: Modified Marsh classification, duodenal biopsies, celiac disease

ÖZET

Amaç: Çöliak hastalığı, buğday, arpa ve yulaf gibi tahıllı gıdalarda bulunan, gluten proteinleri ile oluşan, toplumun %1 inden fazlasının etkilendiği, ince barsak enflamasyonu ile karekterize otoimmün bir hastalıktır. Çöliak hastalığında mukozal hasar, otoimmün kompenenti olan, aynı zamanda T hücre aracılı, kronik enflamatuvar hastalık olarak kabul edilen, dinamik bir süreçtir. Bu çalışmanın amacı, 10 yıl içerisinde bölümümüze gelen duedonal biopsilere Modifiye Marsh Klasifikasyonunu uygulamaktır. Bu çalışmanın amacı, daha önce tespit edilemeyen düşük dereceli çöliak olgularının ortaya çıkarılmasıdır.

Materyal ve Metod: 467 biopsi alındı ve 2 patolog tarafından değerlendirildi (2001 ile 2011 yılları arasında Çukurova Üniversitesi Tıp Fakültesi, Patoloji anabilim dalında tanıları konuldu). Her bir örnek daha önce rapor edilen tanılar göz önüne alınmaksızın yeniden değerlendirildi.

Bulgular: Modifiye Marsh Klasifikasyonuna göre 48 olgu Tip 1 çöliak tanısı konuldu. Modifiye Marsh Klasifikasyonuna göre 6 olguya Tip 2 çöliak tanısı konuldu. Toplam 11 olguya Modifiye Marsh Klasifikasyonuna göre Tip3a çöliak tanısı

konuldu. Toplam 5 olguya Modifiye Marsh Klasifikasyonuna göre Tip 3b çöliak tanısı konuldu. Toplam 6 olguya Modifiye Marsh Klasifikasyonuna göre Tip 3c çöliak tanısı konuldu.

Sonuç: Bu çalışma, Modifiye Marsh Klasifikasyonunun klinik şüpheli olgularda duedonal biopsilerin değerlendirilmesinde önemli bir standartizasyon getirdiğini, erken olguların değerlendirilmesinde çok yararlı olduğunu ortaya koymuştur.

Anahtar Kelimeler: Modifiye Marsh klasifikasyonu, duodenal biyopsi, Çöliak hastalığı

INTRODUCTION

Celiac is an autoimmune disease caused by gluten proteins which can be found in multi-grain food like wheat, barley and oat. The disease affects more than 1% of population and characterized by intestinal inflammation[1-2]. Considering the general population, it is reported that the genetic component of the diseases has a strong effect[3]. DQ2 and/or DQ8 molecules can be seen in nearly 97% of all celiac disease events[4]. Other than clinical symptoms such as, cramp like abdominal pain, vomiting, diarrhea[5], Celiac disease can show itself as an iron deficiency, anemia and osteoporosis like symptoms[6].

In suspicion of celiac disease, serological tests should be made before duodenal biopsy[6]. The serological tests of IgA tissue transglutaminase (tTG) and IgA endomysial antibodies (tEMA) are shown to be highly specific and sensitive to diagnosis of celiac disease. IgA (tTG) test should be the first choice. If the result of this test is uncertain, then Ig-EMA test is recommended[7].

The major histological changes in Celiac disease are flattening of villous, atrophy[8-11], crypt hyperplasia, degeneration of enterocyte, intraepithelial lymphocytosis, increase of eosinophils in lamina propria and increase of mononuclear inflammatory cells. Therefore the pathological report should include the atrophy of villous and the degree of crypt hyperplasia beside the intraepithelial lymphocyte count[5]. There are studies to provide standardization and scoring of histological findings using modified Marsh classification Table 1.

In celiac disease, mucosal damage is a dynamic process. It is shown that it has autoimmune components. It is also T-Cell mediated and can be categorized as a chronic inflammatory disease. The mucosal lesions in Celiac disease are normally patch shaped. Therefore, in order to make a diagnosis, at least 4-6 biopsies should be taken. Effects of the size of the biopsy forceps on diagnosis are found to be very little. Yet for pathologists, big biopsies are more efficient since their orientation is easier.

The purpose of this study is to make Modified Marsh Classification (MMC) of the duodenal biopsies that came to our clinic in 10 years. The study deals with reassessment of all events and uncovering the low graded events that were not diagnosed.

MATERIALS and METHODS

During the ten years period, 467 biopsies were diagnosed between 2001 and 2011 at the Cukurova University, Faculty of Medicine, and Department of Pathology. These biopsies were taken and analyzed by two pathologists. The causes for lymphocytic duodenitis (increased intraepithelial lymphocytosis in normal villous structure); helicobacter pylori, drugs, autoimmunity and cases of food allergy were ruled out. Each sample was reevaluated without taking the previous reports into consideration and scored by using MMC (Table 1). At the same time, 5 examples with active chronic duodenitis and eosinophyl leukocyte infiltration were applied with CD 117 (c-kit) with immunohisto-chemistry searching for giardia.

Table 1. Scoring of histological findings using MMC.

	Type 0	Type 1	Type 2	Type 3a	Type 3b	Type 3c
No of İEL	<40	>40	>40	>40	>40	>40
Crypt	Normal	Normal	hypertrophy	hypertrophy	hypertrophy	hypertrophy
Villous	Normal	Normal	Normal	mild atrophy	Obvious atrophy	Absent

RESULTS

In the biopsy samples of 467 cases the average age was 55. No repeat biopsies are taken from the same patient. According to MMC, a total of 48 cases (21 Male, average age: 48 and 27 Female, average age: 42) were diagnosed as Type 1 celiac (Figure 1). These diagnoses were previously made as active chronic duodenitis (21 cases), chronic duodenitis (18 cases), non-specific duodenitis (2 cases), normal duodenum mucosa (5 cases). Clinical information of the samples' did not include the cases' serological findings. However, in every sample, the pre-diagnosis was celiac disease. Also one of the cases, diagnosed as Type 1 celiac according to MMC, was reported as lymphoplasmacytoid inflammation while another case was reported as hemorrhage and congestion (Table 2).

Total of 6 cases (4 Male, average age: 60 and 2 Female, average age: 52) according to MMC was diagnosed as Type 2 celiac (Figure 2). These cases were previously diagnosed as active chronic duodenitis (3 cases) and chronic duodenitis (3 cases) (Table 2).

Total of 11 cases (6 Male, average age: 42 and 5 Female, average age: 46), according to MMC, was diagnosed as Type 3a (Figure 3). These cases were previously diagnosed as active chronic duodenitis with focal villous atrophy (3 cases), duodenitis with focal villous atrophy (2 cases), and villous atrophy with Lympho Plasmacytoid Cell Infiltration (LPCI) (5 cases). Only one case was previously diagnosed as active chronic duodenitis.

Total of 5 cases (1 male, avr. age: 69 and 4 female, avr. age: 37), according to MMC was diagnosed as Type 3b celiac (Figure 4). They were previously diagnosed as common villous atrophy with LPCI (1 case), active chronic duodenitis (1 case), chronic duodenitis with villous flattening and compatibility with gluten enteropathy (2 cases), villous atrophy with LPCI (1 case).

Total of 6 cases (5 female avr. age: 28 and 1 male avr. age: 21) according to MMC was diagnosed as Type 3c celiac (Figure 5) They were previously diagnosed as active chronic duodenitis with total villous atrophy (3 cases), total villous atrophy with compatibility with gluten enteropathy (1 case), LPCI with villous atrophy (1 case) and villous atrophy (1 case).

Duodenal biopsy with much eosinophilic leukocyte infiltration in tunica propria was reevaluated for giardiasis. In 5 cases suspected interference was seen and CD117 was applied to search for giardia. In test results of two cases CD117 (+) giardiasis was found (Figure 6).

Table 2. According to the Modified Marsh Classification, re-distribution of 76 cases

	Active chronic duedonitis	Chronic duedonitis	Non specific duedonitis	Normal duedonal mucosa	LPCI	Conjestion and edema	Focal villous atrophy+ active chronic duedonitis	Focal villous atrophy +chronic duedonitis	Villous atrophy+ LPCI	Diffuse villous atrophy + LPCI	Total villous loss+active chronic duedonitis	Villous atrophy Hyperplasia chript
Type 1	21	18	2	5	1	1						
Type 2	3	3										
Type 3a	1						3	2	5			
Type 3b	1	1								1		2
Type 3c									1		3	2

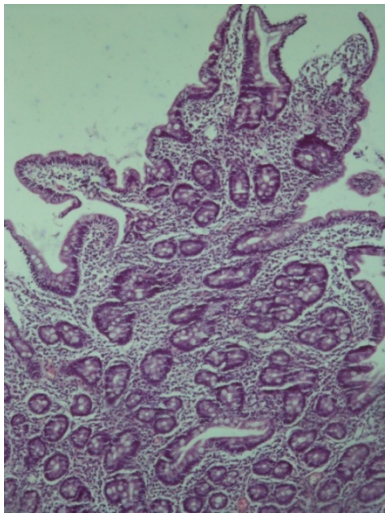

Figure 1. Celiac disease or Type 1 according to MMC, x100

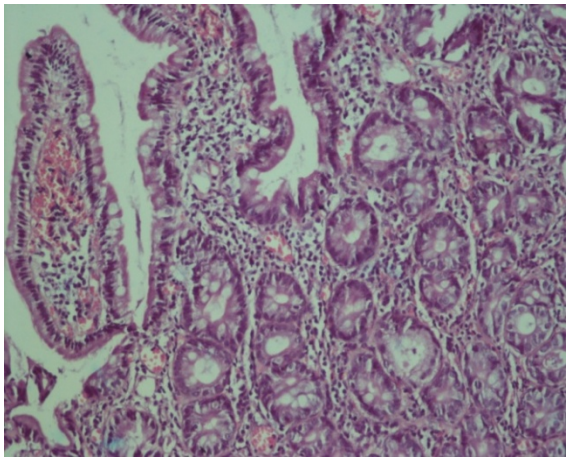

Figure 2. Celiac disease or Type 2 according to MMC, x200.

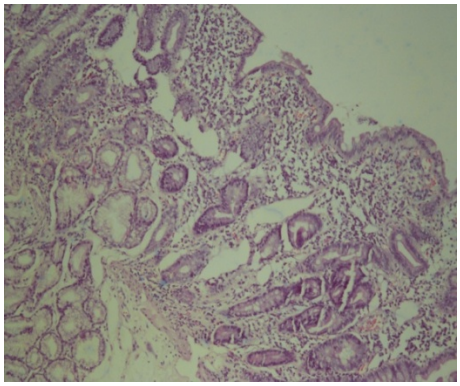

Figure 3. Celiac disease or Type 3a according to MMC, x100

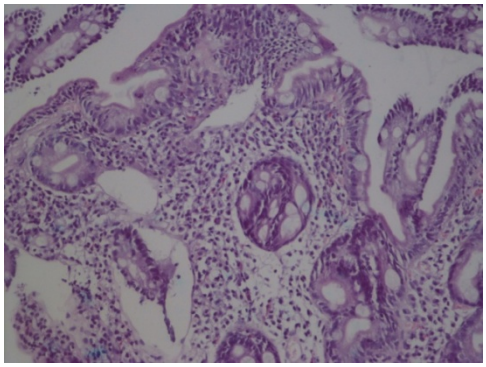

Figure 4. Celiac disease or Type 3b according to MMC, x200

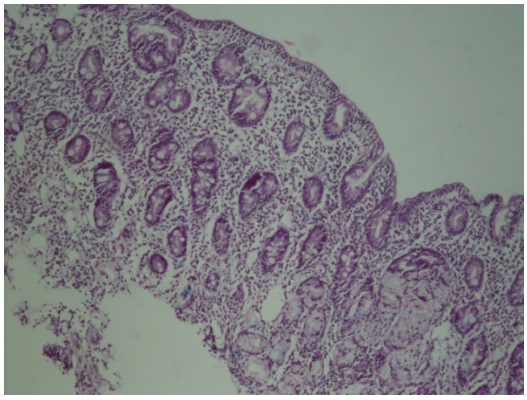

Figure 5. Celiac disease or Type 3c according to MMC,x100

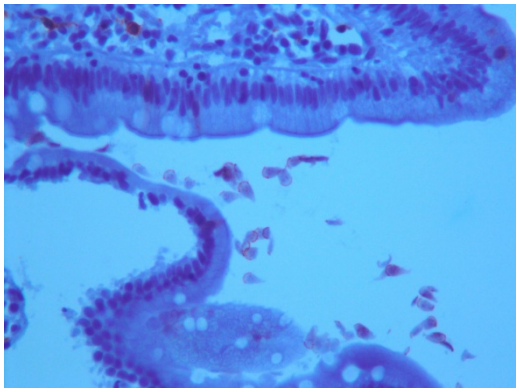

Figure 6. Giardiasis, in duodenal mucosa, x400

STATISTICS

In this study, a total of 467 cases were reevaluated according to MMC. 76 of them (Type 1:48, Type 2:6, Type 3a:11, Type 3b:5, Type 3c:6) were rediagnosed according to MMC. Descriptive statistics were used. Diagnosis counts and related percentages were given in Table 2 and Table 5, respectively.

Table 5. The percentages of diagnosis

	Active chronic duedonitis	Chronic duedonitis	Non specific duedonitis	Normal duedonal mucosa	LPCI	Conjestion and edema	Focal villous atrophy+ active chronic duedonitis	Focal villous atrophy +chronic duedonitis	Villous atrophy+ LPCI	Diffuse villous atrophy + LPCI	Total villous loss + active chronic duedonitis	Villous atrophy at Hyperplasia chript
Type 1	27.6	23.7	3.6	6.3	1.3	1.3						
Type 2	3.9	3.9										
Type 3a	1.3						3.9	2.6	6.5			
Type 3b	1.3	1.3								1.3		2.6
Type 3c									1.3		3.9	2.6

DISCUSSION

Clinically, in most cases, serologically and histologically, diagnosing celiac disease is very easy. If serologically, celiac autoantibodies IgA-TTG and EMA are high-titer positives with typical symptoms then finding Celiac disease in intestine mucosa is nearly 100%[17]. Celiac disease can generally be seen on the proximal of intestine. Morphological findings can be seen up to jejunum[18]. In duodenal biopsy, flattening of mucosa is typical. However in the early stages of the disease mucosa may appear to be normal. It is recommended that biopsy is taken twice from both duodenum's first and second parts. As there is no standard on how to take a biopsy, samples were generally taken from the second or the third part and normally includes only two tissue samples.

In the previous studies, it was reported that the location of the biopsy (duodenum's second, third and fourth part), using standard forceps rather than jumbo forceps, does not affect the diagnosis of celiac disease[19]. Latest studies show that taking biopsy especially after the second part of duodenum from bulbous is very much important. They also emphasize on the importance of biopsy count rather than the size of the biopsy forceps.

In the literature it is said that the ideal biopsy count should be 4 to 6[6]. In the biopsies, general duodenum structure, villous-crypt ratio and crypt hyperplasia are evaluated. Therefore, in the biopsy samples, the need is for 3-4 villous that can be viewed. Normally, villouses are thin. Normal crypts/villous ratio is known to be 3/1 - 5/1[20]. In literature, 2/1 and even 1/1 ratios are reported as normal[21-22].

At mucosal surface, it is seen that increase in mitosis with a high turnover results in first as villous blunting, then as crypt hyperplasia and then as deepening crypts. Krypt hyperplasia is stimulated by intraepithelial T lymphocytes and forms the typical appearance of the celiac disease[8].

The intraepithelial lymphocyte count in villous epithelia is very important for celiac disease. The density of the lymphocytes is usually calculated with the count of lymphocytes in 100 enterocyt cytoplasm. However, the important point is that there is a diffuse distribution in both the edges and the tips of the villous. The intraepithelial lymphocyte, seen in just the tips of the villous, is not a typical case for a celiac disease[23].

At MMC it is emphasized that traditionally intraepithelial lymphocyes (IEL) count should be less than 40. However, in the biopsies that were taken from the distal intestine with Crosby capsule show that IEL count is more than 40 in normal duodenum[24].

Because of these reasons, diagnosing celiac disease in early-stages from the biopsy samples is a hard task. The unstable structure of the villous, frequently seen in biopsy artefacts, changes with ethnicity. Besides, the insufficiency of biopsy sample count and the difficulties of sample localization complicate the reporting task. Therefore, it may not contribute to the diagnosis of the case that had samples with different diagnoses.

Table 3. Celiac disease mimicking other diseases

Hiperplasia Infectious chript gastroenteritis
Giardiasis
Radiation enteropathy
AIDS enteropathy
Crohn's disease
Eosinophilic gastroenteritis
Zolinger ellison syndrome
Dermatitis herpetiformis
Viral enteritis, lymphoma, ischemic enteritis
Drug effects, autoimmune enteritis, tropical sprue
Collagenous colitis
Bacterial overgrowth, Hypogamaglobilinemy
Graft versus host reaction

Marsh and Oberhuber have made the first celiac classification. It was later on reformed by Corazza and Villianacci's simplifications, forming the MMC known today. In Table 1, atrophic and non atrophic degrees split into groups in themselves. In this classification, the main issue is the changes of villous structure, crypts and intraepithelial lymphocytes. With MMC, it is possible to reduce the number of the diagnostic categories thereby becoming more useful to clinician. With this scoring technique, the concordance between pathologists is increased. Still, rarely some celiac cases might be hard to diagnose. In these cases, HLA genotyping might be necessary as suggested by Maki et al and

McGowan at al[25,26]. In a study reported by AD Hoper and Arc, it was reported that celiac disease could not be determined serologically but it is necessary to take biopsy for the clinical cases with suspicion of celiac disease[27]. And the doctor should keep in mind the other diseases that imitate celiac disease (Table 3).

In addition, it is necessary to consider that the intensity of the histopathology findings is not always correlated with clinical findings and symptoms. Even cases with intense villous atrophy Celiac disease could be asymptomatic. Furthermore, the intraepithelial lymphocytosis can be seen on many diseases (Table 4). In the sections that are 3 to 4 micron in thickness, if there is over 20 lymphocytes in 100 enterocytes then it can be considered as a pathological finding.

Table 4. The other diseases that intralenfositic lymphocytosis

Helicobacter pylori , celiac disease
Giardiasis
HIV enteropathy, autoimmune enteropathy
IgA deficiency lymphocytic enteritis
The blind loop syndrome of NSAID medicines
Tropical sprue
Hypogamaglobilinemy

In this study, immunohistochemical studies were made to determine lymphocytes that are a little above 20 in 100 enterocytes[28]. In cases with a slight lymphocytosis, immunohisto-chemical method with CD 3 study is recommended to determine T lymphocytes.

Giardia Lambdia is a non life-threatening disease that causes loss of fluid with diarrhea[29]. Histopathologically the mucosal changes in the intestine could change from normal mucosa to partial villous atrophy. Trofozoids can generally be found at duodenal mucosa (82.5%), jejunal mucosa (2.1%), gastric antral mucosa (8.7%), ileal mucosa (12.1%) and colon (0.4%)[30]. In this study, 5 samples with eosinophilia was examined. Immunohistochemical method with CD117 (c-kit) was used on the samples. Two of the samples were diagnosed with giardia positive (+).

In this study duodenal biopsies that were previously examined were re-examined with MMC. In the previous examination, only 20 celiac cases were identified. With the re-examination, a total of 76 previously unidentified or missed early diagnosis cases of celiac disease have been identified by MMC. This study could be handicapped due to lack of results of serological examination for most of the cases. The study was retrospective as the number of duodenal biopsies was generally 2. They were taken from second and third parts of duodenum.

In spite of all there handicaps, the results of reexamining 467 cases using MMC reveals 76 cases gained and this corresponds to 15.9% of total cases thereby making this study a successful and meaningful one. The results of this study have shown that MMC is a very important standardization for assessment of suspicious duodenal biopsies and especially early case examinations.

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