HLA Typing and Histopathologic Features of Patients with Celiac Disease-A Retrospective Study

Çöliak Hastalığı Olan Hastalarda HLA Tiplendirme ve Histopatolojik Özellikler

HLA Subtypes and Histopathology in Celiac Disease

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Özet

Amaç: Çöliak Hastalığı, beslenme intoleransının en sık görülen defektlerinden birisidir. Glutene artmış bir duyarlılık vardır. Multifaktöriyel olarak kalıtılmaktadır, bu yüzden çevresel ve genetik etkenler hastalığı değerlendirmede önemlidir. Özellikle DQ2 ve DQ8'i kodlayan spesifik insan lökosit antijenlerinin varlığı, çöliak hastalığı tanısını koymada önemlidir. Bu çalışmada, kronik diyare, karın ağrısı ve benzeri semptomlarla gelen hastalarda çöliak hastalığı açısından insan lökosit antijeni allel dağılımını belirlemek amaçlanmıştır. Gereç ve Yöntem: Laboratuarımıza başvuran 40 hastanın insan lökosit antijenleri prevalansı, polimeraz-zincir reaksiyonu- sekans spesifik primer metodu ile incelenmiştir. Hastaların Marsh sınıflandırmaları da, bir patolog tarafından değerlendirilmiştir. Bulgular: Hastaların %95'inde insan lökosit antijeni saptanmıştır. En sık görülenler, DQA1*0501 ve DQB1*0201, en az görülen ise DQA1*0501 ve DRB1*04 kombinasyonu idi. Marsh sınıflandırmasına göre Grade 2 Marsh Tip 4, hipoplastik tip en sık olarak saptandı. Tartışma: Çöliak hastalığının progresyonunda ve hastalığa yatkınlığın belirlenmesinde, insan lökosit antijenleri tiplendirmesi klinisyenler için yardımcı olacaktır.

Anahtar Kelimeler

Çöliak Hastalığı; Histopatoloji; Marsh Sınıflandırması

Abstract

Aim: Celiac disease is the most common defect of nutrition intolerance. There is an increased sensitivity to the glutene. It is inherited multifactorial, because of this, genetic and enviromental factors are important in the evaluation. The existence of specific human leukocyte antigen alleles coding especially DQ2 and DQ8 are important at the diagnosis of celiac disease. In this study, it is aimed to determine human leukocyte antigens allel distribution for celiac disease in patients with chronic diarrhea, abdominal pain and similar symptomes. Material and Method: The prevalance of human leukocyte antigens of 40 patients applied to our laboratory were searched using polimerase chain reaction-sequence specific primer method. Marsh classification of the patients were also performed by a pathologist. Results: We determined human leukocyte antigens in 95% of the patients. The most common antigens were DQA1*0501 and DQB1*0201. The combination of DQA1*0501 and DRB1*04 was the least. According to Marsh classification, Grade 2 Marsh Type 4 hypoplastic was the most common type. Discussion: The human leukocyte antigen typing is helpful for the clinicians at the progression of the celiac disease and at the prediction of the tendency to the disease.

Keywords

Celiac Disease; HLA Typing; Histopathology; Marsh Classification

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Introduction

Celiac disease or gluten enteropathy (CD;MIM 212750) is a type of autoimmune malabsorption, improving by impairment of absorptive area in the small bowel. It is the most common defect of nutrition intolerance (1%) [1,2]. The disease is common in Europe, particularly in Finland, Italy, Ireland and Sweden where the reported prevalence is as high as 0.3–1% [3]. CD is more common in females according to males [4].

The main problem in celiac disease (CD) is the increased sensibility to a protein, called gluten. Gluten is the component of wheat which includes gliadine. When small intestine is exposed to gluten, this causes small-intestinal mucosal inflammation and morphological damage with symptoms of malabsorption and recurrent diarrhea attacks [5,6]. The symptomes of CD can begin at any age. The treatment is the elimination of gluten from the diet. The disease affects several organ systems including skin, liver, nervous system, bones, reproductive system and endocrine systems. Diarrhea, abdominal pain and weight loss are the main symptomes [7,8]. Dermatitis herpetiformis, a pathognomonic skin eruption, occurs in 10-20% of patients with celiac disease [7]. In last decades, the small intestinal biopsy with typical pathological lesions was considered as the gold-standard for the diagnosis of CD [5]. The new European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria is published in 2012 and recently the diagnosis of CD, according to this new criteria, is based on: gluten-dependent symptoms, Human Leukocyte Antigen (HLA) typing (presence of HLA-DQ2 and/or DQ8), CD-specific antibodies levels (anti-tissue transglutaminase type 2 (anti-TG2), anti-endomysium (EMA) and characteristic histological changes [8].

CD is one of the most common genetic disorders with a prevalance of 1-2.67%, recently [9]. There is an increased risk (5-10%) of CD in first-degree relatives of CD patients [10]. CD has a multifactorial inheritance [3]. Due to the multifactorial inheritance of the disease, twin studies are important at the evaluation of genetic and enviromental components in the tendency to the disease. The existence of specific HLA alleles especially coding DQ2 and DQ8 are important in the diagnosis of CD. Screening the family members of a CD patient, can be helpful for the diagnosis of new CD patients [11,12].

Human Leukocyte Antigen (HLA) system is the major CD-predisposing genetic factor. Nearly 90% of celiac patients present HLA-DQ2 heterodimers, which also called as DQ2.5. These are encoded by DQA1*05 and DQB1*02 alleles [3]. DQ2.5 negative patients (nearly 5-10%) carry DQ8 heterodimers in combination with DR4 haplotype (DRB1*04). The HUGO Gene Nomenclature Committee has declared HLA-DQA1 and HLA-DQB1 class II genes as CELIAC 1 [3]. HLA-DQ2 or HLA -DQ8, or both, are found in approximately 40% of the general population, but in more than 99% of patients with celiac disease. If these DQ predisposing alleles are absent, celiac disease is highly unlikely. The risk of disease is higher among DQA1*05/DQB1*02 heterodimer carriers than individuals homozygous for DQB1*02 [13,14]. The serological diagnosis of CD is based on the detection of anti-tissue transglutaminase A/ B (anti-tTG A/B) and endomysial antibody (EMA). Especially, EMA specificity is very high (98-100%), so this test is assigned specific for the diagnosis of CD. In this study, we described a series of patients with CD and the serologic, genetic and histologic profiles that characterized these patients.

Material and Method

A total of 40 patients, 36 women and 4 men, applied to our laboratuary, between 2012-2013, because of symptomes like chronic diarrhea, abdominal pain and weight loss, are included to this study. 28 patients had positive anti-tissue transglutaminase antibody (anti-tTG), 26 patients had endomysial antibody (EMA), 32 patients had high level of anti-gliadine antibodies. These patients had histologically confirmed diagnosis of CD and were analysed genetically for CD. Genomic DNA extraction from white blood cells was performed using the QIAamp® DNA Blood Kit (QIAGEN® Inc., Valencia, CA). The prevalance of class II HLA antigens in CD was investigated by using Olerup SSP® (Hasselstigen, Sweden) kit and the method was Polimerase Chain Reaction (PCR)- Specific Sequence Primer (SSP). Informed consent of the patients were taken. The clinical symptoms at presentation were collected from the medical records. The study was performed according to the guidelines of the local medical ethics board. Histological evaluation was performed by the biopsies using upper endoscopy. The biopsies were evaluated by a single experienced pathologist who didn't know the patients' clinical data and used the Marsh classification, as modified by Oberhuber [15,16]. The duodenal bulb and the distal duodenum were scored separately, but the final Marsh score for each patient was graded according to the most affected site (highest Marsh score).

Results

The mean age of the patients was 42.45 (22-61years). The biopsies of these 40 patients were sent to pathology laboratuary. These patients' initial diagnosis was gluten enteropathy and laboratuary findings supported this diagnosis. Hematoxylin and eosin stained slides of 40 patients were re-evaluated by an experienced pathologist retrospectively. The biopsies which were compatible with gluten enteropathy were classified again according to Marsh-Oberhuber classification. The image of villi, the increase of intraepithelial lymphocyte (30 lymphocyte/100 erythocyte or 5 lymphocytes in one villus) and the structure of crypts were evaluated. The Marsh classification of 15 patients were Marsh type 4 hypoplastic type, 9 patients were Marsh destructive type 3c, 7 patients were Marsh destructive type 3b, 4 patients were Marsh destructive type 3a, 4 patients were Marsh hyperplastic type 2 and 1 patient was Marsh infiltrative type 1 (Table 1). In hypoplastic type 4, there were severe atrophy, smooth surface, atrophic crypts and more than 40 intraepithelial lymphocyte infiltration at villi. In destrictive type 3a, there were mild coarsening, hypertrophic crypts and more than 40 intraepithelial lymphocyte infiltration at villi. In destructive type 3b, there were mild coarsening, hypertrophic crypts and more than 40 intraepithelial lymphocyte infiltration at villi. In destrictive type 3c, there were severe coarsening, straightness, hypertrophic crypts and more than 40 intraepithelial lymphocyte infiltration at villi. In hyperplastic type 2, there were hypertrophic crypts and more than 40 intraepithelial lymphocyte infiltration at villi, the structure of villi was normal. In infiltrative type 1, there was more than 40 intraepithelial lymphocyte infiltration

Table 1. Number of patients according to HLA genotypes and Marsh classification

HLA Genotype	Number of patients	Grade II Marsh Type 3c, Destructive type	Grade II Marsh Type 3b, Destructive type	Grade II Marsh Type 4, Hypoplastic type	Grade II Marsh Type 2, Hyperplastic type	Grade I Marsh Type 1 Infiltrative type	Grade I Marsh Type 4, Hypoplastic type	Grade I Marsh Type 3b, Destructive type	Grade I Marsh Type 3a, Destructive type	Grade I Marsh Type 3c, Destructive type	Grade I Marsh Type 2, Hyperplastic type
DQA1 05-DQB1 02	21	3	4	5	3		1	1	2	2	
DQA1 05	5	1		3					1		
DQA1 05-DRB1 04	5	1		2					1		1
Negative	2			1			1				
DQA1 05-DQB1 02-DRB1 04	4	1		1		1		1			
DRB1 04	2	1	1								
DQB1 02	1			1							

at villi. The structures of crypts and villi were normal. In preinfiltrative type 0, the structures of villi and crypts are normal, the number of intraepithelial lymphocytes is less than 40 and there is normal duodenal mucosa. There was no preinfiltrative type 0 stage in our patients.

For the typing of class II HLA antigens, HLADQA1*0501, HLADQB1*0201, HLADRB1*04 and HLADR8 allelles were analysed. HLADQA1*0501 and HLADQB1*0201 allelles were determined in 21 (52.5%) patients. HLADQA1*0501 allele was determined in 5 (12.5%) patients, association of HLADQA1*0501 and HLADRB1*04 allel in 5 patients (12.5%), HLADQB1*0201 allel in 1 (2.5%) patients, HLADQA1*0501, HLADQB1*0201 and HLADRB1*04 association in 4 (10%) patients, HLADRB1*04 allel in 2 (5%) patients. 2 (5%) patients were negative for these allelles (Table 1). Association of HLADQA1*0501 and HLADQB1*0201 allelles were the most seen alleles in women while it was HLADQA1*0501, HLADQB1*0201,HLADRB1*04, allels in men. The most common Marsh classification was grade II Marsh type 4 hypoplastic and grade II marsh type 3b destructive type, respectively, at HLADQA1*0501 and HLADQB1*0201 positive patients.

EMA was positive in 26 patients (65%), anti-tTG was positive in 28 patients (70%). The level of anti-gliadine antibodies IgA and IgG were high in 32 patients (32%)

Discussion

Genetic risk profiles for CD would be helpful in clinical practice for predicting disease susceptibility and progression [17]. Most of the CD patients have HLA DQ2 and DQ8 alleles which are responsible from 40% of genetic susceptibility to CD [9]. It has been indicated that the prevalance of DQ2 and DQ8 genes differs according to the different populations [18]. In our study, the most seen alleles were HLADQA1*0501 and HLADQB1*0201 allels (52.5%), respectively HLADQA1*0501 allel and HLADQA1*0501, HLADRB1*04 allels were the same (12.5%). This is compatible with the literature, in other words it can be said that the frequency of the most common allels in Turkish population is towards DQ2. In a study in Libya, it has been reported that DQ2/DQ8 genotype is more common than in European countries [18]. Especially the effect of DQB1*0201 is great. While to carry the two copies of DQB1*0201 is associated with increased disease risk, it has no effect on the early age onset and diagnosis or on the estimation of the severity of the disease [19]. In the recent studies, it has been showed that female patients often carry DO2.5 and/or DQ8 molecules, but males are oftenly negative for DQ2.5/DQ8 [20]. In our study, the number of male patients is low, so we couldn't detect this finding.

The new CD guidelines suggest two revolutionary and major respects for the diagnosis of CD. One of them is the role of serological tests in the diagnostic process of symptomatic cases, the other one is the determination of HLA DQ2/DQ8 in the diagnosis of asymptomatic cases who are at risk for CD. If tests for EMA antibodies and HLA typing are positive, then the diagnosis of CD is confirmed [21]. In our study, EMA was positive in 26 patients (65%) and HLA was positive in 38 patients (95%).

Lately, European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published some new diagnostic criteria; anti-tTG levels 10-fold high the upper limit of normal, positive EMA antibodies and specific HLA haplotype. These criteria simplify the diagnosis of CD [4]. In some selected patients with symptomes of CD, small bowel biopsy can be avoided by these criteria [4,22,23]. The quality of serological tests and the rare usage of HLA testing are the limitations at the diagnosis of CD without a small bowel biopsy. So, both of these tests are important tools in the diagnosis if they are performed at qualified laboratories [4].

Polimerase Chain Reaction- Sequence Specific Primer (PCR-SSP) is an accurate HLA typing technique with high sensitivity, specificity and reproducibility. The method is rapid and inexpensive [24]. In a study in USA, 10191 patients participated to the study. Homozygosity for HLA-DQ2 heterodimer dramatically elevated CD risk in this at-risk population. These data demonstrate that knowledge of a patient's HLA-DQ genotype is a powerful tool for stratifying risk. HLA haplotyping might be of benefit in testing infants never exposed to gluten, young children who might not make antibodies, patients with indeterminate serologies or biopsies, relatives of biopsy-diagnosed individuals, patients

with IgA deficiency and patients on a self-imposed glutenfree diet (GFD) unwilling to undergo gluten challenge. This approach could reduce cost and psychological burden within families [25]. HLA typing is preferred by clinicians especially in uncertain cases or in at-risk groups. It is also used for the relatives of CD patients. HLA typing has not a diagnostic importance, but if it is negative, the diagnosis of CD should be left. So if there is a positive result, this implies that there is a genetic predisposition for celiac autoimmunity and the other tests (serological and if necessary small bowel biopsy) should be performed [3]. In our study, HLA typing was performed. The results were negative in only 2 of 40 patients. This is important for clinicians to diagnose celiac disease. The clinicians diagnose the patients as CD according to HLA typing and sometimes they can be avoided from small intestinal biopsy [3].

In conclusion, CD is a common multifactorial disease and specific HLA-DQA1 and HLA-DQB1 alleles are important in the genetic predisposition. A positive test shows the genetic susceptibility, but doesn't mean the disease development. But, a negative test is more significant, because in the absence of specific HLA alleles, CD rarely occurs. The prevalance of DQ2 and DQ8 genotypes is extremely high in Turkish population as in European countries. HLA typing in individuals at high risk for CD will be helpful at the diagnosis and it can prevent redundant medical interventions. In future, studies covering wide populations about this subject will be quite rather useful for the diagnosis and treatment of the disease. We also intend to enlarge our study by adding more patients diagnosed as CD and also their relatives to our study group.

Competing interests

The authors declare that they have no competing interests.

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