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Prevalence of HLA-DQ*02 and HLA-DQ*08 in Patients with Celiac Disease in Eastern Anatolia and the Diagnostic Role of HLA-DQ*02 and HLA-DQ*08 Genotyping

Çölyak Hastalığının Tanısında HLA DQ*02,HLA DQ*08 Genotiplemesinin Rölü ve Çölyak Hastalığı Olanlarda HLA DQ*02,HLA DQ*08 'in Sıklığı

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Abstract

Introducton: Celiac disease (CD) is diagnosed with serological tests and small bowel biopsy. There is a strong link between CD and human leukocyte antigens (HLA). In this study, we aimed to determine the role of HLA alleles DQ*02 and DQ*08 in the diagnosis of pediatric CD patients and to determine the prevalence of these alleles in the population.

Materials and Methods: The study included 72 school-aged celiac patients diagnosed according to serology and small bowel biopsy results, and a control group consisting of 70 unrelated individuals with no systemic disease. HLA-DQ*02 and HLA-DQ*08 typing was done using the sequence-specific primer (PCR-SSP) method.

Results: The mean age of the CD patients included in the study was 10.06±2.10 years. HLA-DQ*02 frequency was significantly higher in the CD group (67%) compared to the control group (17%) ($p<0.001$). HLA-DQ*08 frequencies did not differ significantly between the patient and control groups (26% and 24%, respectively; $p>0.05$).

Conclusions: Genetic risk profiles in CD are helpful for predicting susceptibility to disease and disease progression. The results of our study showed that the prevalence of HLA-DQ*02 was higher among CD patients than healthy individuals, and it was higher than the prevalence of HLA-DQ*08. Our study further supports the link between HLA-DQ*02 and increased risk of disease.

Keywords: Celiac disease, HLA-DQ*02, HLA-DQ*08

Öz

Giriş: Çölyak hastalığı serolojik testler ve ince barsak biyopsisi ile konur. Çölyak hastalığının insan lökosit antijenleri (HLA) ile güçlü bir bağlantısı vardır. Bu çalışmada pediatrik çölyak hastalarının HLADQ*02 ve DQ*08 alleleerinin çölyak hastalığının teşhisindeki rölünün ve populasyondaki sıklığının belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya serolojik testler ve ince barsak biyopsisi sonuçlarına göre tanı koyulan okul çağındaki 72 Çölyak hastası ile herhangi bir sistemik hastalığı bulunmayan ve akraba olmayan 70 sağlıklı birey kontrol grubu olarak çalışmaya dahil edildi. Sekansa spesifik primer (PCR-SSP) yöntemiyle HLA DQ*02 ve HLA DQ*08 tiplemesi yapıldı.

Bulgular: Elde edilen bulgulara göre çalışmaya alınan çölyak hastalarının yaş ortalaması 10.06±2.10 yıldır. Çölyak hastaları (%67) ile kontrol grubu (%17) karşılaştırıldığında hasta grubunda HLA DQ*02 pozitifliğinin yüksek olduğu görüldü ($p<0.001$). Yine hastalarda (%26) ile kontrol grubu (%24) karşılaştırıldığında hasta grubunda HLA DQ*08 pozitifliğinde istatistiksel olarak anlamlı bir fark saptanmadı ($p>0.05$).

Sonuç: Çölyak hastalığındaki genetik risk profilleri, hastalığa yatkınlığın tahmininde ve hastalığın progresyonunda klinik tecrübe elde edilmesine yardımcıdır. Sonuç olarak, çalışmamızda çölyak hastalarında HLA DQ*02 frekansının HLA D*08 frekansından ve sağlıklı bireylerden daha yüksek olduğu görülmektedir. Özellikle HLA DQ*02'nin artmış hastalık riskiyle olan ilişkisi çalışmamızda bir kez daha belirtilmiştir.

Anahtar Kelimeler: Çölyak hastalığı, HLA-DQ*02, HLA-DQ*08

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Introduction

Celiac disease (CD) is an autoimmune disease that occurs in genetically predisposed individuals due to sensitivity to gluten found in grains. It is usually manifests with malabsorption and characteristic lesions in the intestines. The disease can be asymptomatic or can cause symptoms such as abdominal pain, bloating, weight loss, and diarrhea. CD has been associated with various conditions such as iron deficiency

anemia; elevated liver enzymes; autoimmune diseases such as type 1 diabetes, autoimmune thyroiditis, and Sjögren's syndrome; osteopenia; osteoporosis; and genetic diseases such as Down and Turner syndromes.[1–2]

Although the reason is unknown, CD is more common in females than in males. The prevalence of CD is increasing worldwide, and varies by population and region. Globally, the prevalence is reported as 0.05–0.1%.[3] In Turkey, studies have indicated a prevalence of 0.47%. The prevalence of CD in Europe has risen to 0.3–1%, with particularly high incidence in Finland, Ireland, Sweden, and Italy.[3-4] The incidence in first-degree relatives is 5–10%. Environmental and genetic factors have a major role in the development of the disease.[3–4]

The human major histocompatibility complex is a family of genes that encodes HLAs, which have a crucial role in defense against foreign pathogens and immune surveillance of tumours.[5]

Strong association between the HLA region and autoimmune disease as been established for over fifty years.[3-6] Association of components of the HLA class II encoded HLA-DRB1-DQA1-DQB1 haplotype has been detected with several autoimmune disease, including rheumatoid arthritis, type 1 diabetes and celiac' disease.[6]

The role played by the HLA-DR/-DQ molecules in exogenous antigen presentation to $CD4^+$ Th cells helps to explain their association with several autoimmune disease. Exogenous peripheral antigens are internalized via antigen presenting cells (APC) and are degraded into 13–18 amino acid residue peptides, preferentially bound by HLA class II molecules, in the increasingly acidic compartments of the endocytic pathway.[6]

HLA class II alleles DQ*02 and DQ*08 have been identified as the genetic basis of CD, and their presence facilitates diagnosis. HLA-DQ*02, HLA-DQ*08, or both can be present. Forty percent of the general population carry one or both of these alleles. However, these alleles are present in 99% of CD patients. HLA class II DQ*02 and DQ*08 allele negativity can rule out the diagnosis. In all populations, the HLA-DQB1*02 and DQB1*08 genotypes are a significant risk factor for the development of CD.[3–7]

There are specific pairs of the allelic variants of *HLA-DQ*A1* and *HLA-DQ*B1* implicated in the etiopathogenesis of CD.

HLA-DQ*A1 and HLA-DQ*B1 are found in the p21 region of chromosome 6. HLA-DQ*A1 encodes the α chain of the HLA heterodimer associated with CD, while HLA-DQB1 encodes the β chain of HLA heterodimers associated with CD.[3-6] The HLA-DQ*02 and-DQ*08 alleles encode heterodimeric structures found on the surface of APC. The DQ*02 and DQ*08 proteins each consist of an α and β chain.[3]

It is known that in the pathophysiology of CD, HLA-DQ*022/DQ*08-restricted T cells recognize gliadin peptides, which leads to production of matrix proteins and cytokines.[8] The resulting mucosal damage triggers an autoimmune response. Another notable element in the pathology of CD is lymphocyte infiltration of the epithelium.[8] This indicates that T cell infiltration affects all parts of the gastrointestinal tract.[8–10]

In addition to cellular immunity, it has been determined that humoral immunity is also stimulated in untreated CD patients.[7] Increased numbers of immunoglobin A (IgA)-expressing plasma cells in the intestinal mucosa have been detected in CD.[7] It has been been reported that IgM production is also significantly increased and that cytokine release is stimulated.[7] With antibody production against extracellular matrix proteins stimulated by gliadin, anti-reticulin (ARA) and anti-endomysial (anti-EMA) antibodies are also increased.[7]

The aim of this study was to determine the role of the DQ*02 and DQ*08 alleles in the diagnosis of CD in pediatric patients and to determine their prevalence in the population.

Materials and Methods

Patients and healthy controls

In the first step of screening patients for CD was assessment of IgA-tTG and total serum IgA. Patients with IgA-tTG positivity were then tested for IgA anti-EMA and HLA-DQ*022 and-DQ*08. Those with IgA deficiency were tested for IgG anti-tTG antibody (IgG-tTG). Small intestine biopsies were taken for histological examination from all patients positive for anti-tTG antibodies and were evaluated for CD according to the Marsh classification. Marsh stage 2 or above was considered necessary for a diagnosis of CD. The study included 72 patients who were diagnosed with CD in the Erzurum Atatürk University Pediatric Gastroenterology department between 2015

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Table 1. Anthropometric data of patients with celiac disease

Patient Data	(N=72)
Mean age	10.06±2.10
Gender M/F	27/45

and 2017. Written informed consent was obtained from the patients' parents. The anthropometric characteristics of the patients are presented in Table 1. Seventy healthy, unrelated individuals who had no systemic disease and were not on any form of medication were included as a control group.

DNA Extraction and Celiac disease-Associated HLA Class II HLA-DQ* Typing:

Peripheral blood samples (2 mL) were collected from each patient and control subject into hemogram tubes. Genomic DNA was isolated with a Qiagen EZ1 (Qiagen, Hilden, Germany) automated DNA isolation device. CD-associated HLA class II allele assay was done using the polymerase chain reaction with sequence-specific primer (PCR-SSP) method (Olerup SSP-DQ Low Resolution).

The reactions were run in a PE 9700 thermal cycler. The resulting PCR products were loaded on to an agarose gel; positivities were documented by viewing under UV and analyses were done using Start Scored software.

Statistical analyses

The SPSS statistical package program (version 17.0) was used for all statistical analyses. The distributions of HLA-DQ*02 and HLA-DQ*08 in the CD and control groups were analyzed using the chi-square method. Differences with p values <0.05 were accepted as significant.

Results

As for the distribution of the DQ*02 and DQ*08 haplotypes in the patient and control groups, the DQ*02 haplotype was more prevalent in CD patients (67%) than controls (17%), while the frequency of the DQ*08 haplotype was similar in the two groups (26% and 24%, respectively) (p=0.0012). The difference in prevalence of the DQ*02 haplotype between CD patients and controls was statistically significant (p<0.001). However, the difference in DQ*08 haplotype frequency between patients and controls was not statistically significant (p>0.05) (Table 2).

Discussion

CD is the result of an immune response that occurs due to a combination of environmental and genetic factors, and can significantly affect the quality of life.[9] Serology testing of the blood includes assessment of anti-gliadin antibodies, anti-EMA, and anti-tTG antibodies.[11]Based on the results of these tests, a small intestine biopsy is performed and a diagnosis is made. Genetic testing is also important in the diagnosis because CD is now proven to be a genetic disease.[12] HLA-DQ*02 and HLA-DQ*08 have been implicated in the genetic basis of CD.[13] They encode receptors that bind gliadin peptides and present them to $CD4^+$ T lymphocytes, which then produce various pro-inflammatory cytokines such as interferon gamma.[14] tTG is an enzyme found in the small intestine that deamidates gliadin peptides, thereby increasing their immunogenicity.[15] The subsequent release of metalloproteinases and other molecules that cause tissue damage lead to crypt hyperplasia and villous destruction.[9–11,12–15]

Several molecular methods are used for HLA tissue typing.[16] The most important of these are PCR-SSP,

Table 2. Frequency of DQ*02 and DQ*08 haplotype among celiac disease patients and controls

Haplotype		CD (n=72)	Control (n=70)	p
DQ*02	Positive	67 (93%)	17 (24.3%)	0.001
	Negative	5 (7%)	53 (65.7%)	
DQ*08	Positive	26 (36.1%)	24 (24.3%)	0.05
	Negative	46 (63.9%)	46 (65.7%)	
DQ*02 + DQ*08	Positive	21 (29.1%)	5 (7.1%)	0.05
	Negative	51 (70.9%)	65 (92.9%)	

reverse dot-blot analysis, real-time PCR, and commercial kits designed for celiac disease diagnosis.

The prevalence of CD was reported to be 1/212 in a comprehensive screen of 2.217 school-aged Turkish children conducted in 2010.[16] When previously diagnosed patients and those who do not consent to biopsy are included, the prevalence rose to 1.7%.[16] A study including children aged 6–17 years conducted in Erzurum in 2005 reported a total of 1.263 individuals and reported the CD prevalence as 1/115. This rate was 1/558 in a Greek study including 2.230 adults, 1/157 in Tunisia, and 1/251 among school-aged children in Spain.[2,16–18]

In their prevalence study including 196 CD patients, Cabrera et al. reported that the DR7-DQ2.2/DR3-DQ2 genotype was associated with high risk.[19] In another study, De Silvestri, et al. determined that the HLA-DQB1*02:01 allele was present in more than 90% of pediatric CD patients.[19,20]

Bonatto et al. reported that 98.4% of 74 CD patients carried the HLA DQ*02 and DQ*08 alleles at frequencies of 79.7% and 8.1%, respectively.[21] In another study, Piccini, et al. identified HLA-DQ*02 antigen in 64% and HLA-DQ*08 antigen in 16.8% of CD patients.[22] One of these two antigens was present in 77.5% of the patients.[21,22] Muniz, et al. observed a high prevalence of HLA-DQ*02 and HLA-DQ*08 in blood donors from São Paulo.[23] Amarapurkar, et al. reported that CD was strongly associated with the HLA-DQ*02 and DQ*08 genotypes in the Indian population and that these genotypes had significant diagnostic value when combined with serology in symptomatic patients.[24]

In another HLA-DQ* study conducted by Karell et al. in France, Italy, Finland, Norway, and England, frequencies of 87–93.7% were reported for HLA-DQ*02, 5–8% for HLA-DQ*08, and 89.4–96.7% for HLA-DQ*02 and/or HLA-DQ*08.[25] Vidales, et al. determined frequencies of 93.4% for HLA-DQ*02, 2.4% for HLA-DQ*08, and 95.6% for HLA-DQ*02 and/or HLA-DQ*08.[26]

In one of the studies conducted in Turkey, Baştürk et al. detected the HLA-DQ*02 allele in 67% and HLA-DQ*08 in 25% of CD patients.[27] Additional studies have also demonstrated high HLA DQ*02 allele frequencies, with HLA-DQ*02 prevalences of 84.7% and 52% reported by Kuloğlu, et al. and Tümer, et al. respectively.[27–29]

The present study analyzed CD patients diagnosed based on serological testing or intestinal biopsy. We evaluated HLA-DQ*02 or DQ*08 allele frequencies in this patient group, and our results corroborate previous reports of high HLA-DQ*02 frequencies. The presence of tissue groups other than HLA-DQ and non-HLA antigens will better facilitate the diagnosis of CD in the future.

Ethics Committee Approval: The study was conducted in compliance with international, national, and institutional regulations. The Ataturk University Medical Faculty Ethics Committee approved the study. All persons provided informed consent prior to inclusion in the study.

Informed Consent: Written informed consent was obtained from the patients' parents.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

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