

# HLA-DQ2/DQ8 frequency in adult patients with celiac disease, their first-degree relatives, and normal population in Turkey

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## ABSTRACT

**Background/Aims:** Celiac disease is an autoimmune, familial disease that results in susceptibility to gluten in cereal and cereal products in genetically susceptible individuals. The aim of the present study was to investigate the presence of HLA-DQ2/DQ8 in patients with celiac disease, their first-degree relatives, and healthy community.

**Materials and Methods:** HLA-DQ2/DQ8 analysis was performed in adult patients with celiac disease  $\geq 18$  years old (94 patients), their first-degree relatives (89 people), and healthy group (102 individuals). Anemia, osteoporosis, and diarrhea were interrogated in the celiac patient group and also anti-tissue transglutaminase, anti-endomysium, and anti-gliadin antibodies were recorded.

**Results:** There was a significant relationship between HLA-DQ2/DQ8 presence in all groups, and the distribution of HLA-DQ2/DQ8 in all groups was different ( $p=0.000$ ). No statistically significant correlation was found between the HLA tissue groups and diarrhea ( $p=0.087$ ), osteoporosis ( $p=0.215$ ), anemia ( $p=1.000$ ), tissue transglutaminase antibodies ( $p=0.295$ ), anti-gliadin antibodies ( $p=0.104$ ), and anti-endomysium antibodies ( $p=0.243$ ) in the celiac patient group.

**Conclusion:** HLA-DQ2/DQ8 can be used to diagnose celiac disease particularly when the tests are useless and to screen first-degree relatives.

**Keywords:** Celiac disease, HLA DQ2, HLA DQ8, Turkish population

## INTRODUCTION

Celiac disease is an autoimmune, familial disease characterized by characteristic lesions in the small intestine and usually causes malabsorption, resulting in susceptibility to gluten in cereal and cereal products in genetically susceptible individuals. Symptoms usually occur in the first 3 years of life when cereal is introduced into the diet. The symptomatic second peak of the disease is seen in the third and fourth decades in adults (1).

Celiac disease affects 0.6% to 1% of the population worldwide (2). In a significant study of 20,190 students aged 6–17 years from various regions of Turkey, the prevalence of celiac disease diagnosed with antibody positivity and biopsy was 0.47% (3).

There are wheat, barley, rye, and less high-molecular prolamins that are not soluble in water than in oats. Gliadin,

which is wheat prolamins, is mainly responsible for immunopathogenesis (1).

Gliadin increases the expression of interleukin-15 by causing degradation in epithelial cells, whereas an increased expression of interleukin-15 activates intraepithelial lymphocytes. During infections or as a result of varying permeability, gliadin interacts with HLA-DQ2 or HLA-DQ8 on the surface of antigen-presenting cells as laminae by the transglutaminase in the lamina propria. It is presented to CD4 T cells via the T cell receptor, which causes cytokine release and tissue damage. Thus, villi atrophy and crypt hyperplasia occur (4).

Of the patients, 95% have HLA-DQ2 and less frequently have HLA-DQ8 expression. Screening of HLA tissue may be performed in close relatives of patients with celiac disease who need risk assessment. It is also thought that

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HLA-DQ2 and HLA-DQ8 negativity are excluded in cases with suspicious histological and serological diagnoses (5).

In the diagnosis of celiac disease, autoantibody and biopsy data are inadequate in some patients. In the pediatric age group, tissue group analysis is used to identify this disease. However, there are no data on this issue since adult patients do not have enough study. Therefore, there is a need for randomized controlled studies on this subject. We believe our study can help compensate for this shortcoming. Although there are a limited number of studies on HLA tissue group analysis and clinical use in the pediatric patient group in the literature, a study is not yet available in the adult age group.

In addition, according to the guidelines of the European Society of Pediatric Gastroenterology-Hepatology and Nutrition, HLA tissue analysis is used in the diagnosis of celiac disease and in the screening of first-degree relatives; however, the guidelines of the American College of Gastroenterology state that there are no data on the routine use of HLA tissue analysis in adult patients.

It has not been evaluated whether the present positivity is secondary to celiac disease or a characteristic of the Turkish society in the study of the frequency and clinical use of HLA antigen in children with celiac disease in Turkey, and the percentage of this HLA antigen is not examined in first-degree relatives and normal population.

We would like to discuss whether HLA antigens are significantly higher in adult Turkish patients with celiac disease, whether this is a specific disease of the Turkish society, and if it is specific to the disease, whether the diagnosis can be made in suspicious cases, or whether it can be used for community screening.

#### **MATERIALS AND METHODS**

Between March 2016 and March 2017, patients who were serologically and histopathologically diagnosed with celiac disease, who were >18 years old, who applied to the Gastroenterology and Internal Diseases Polyclinics of Eskişehir Osmangazi University Hospital, their first-degree relatives, and volunteers who participated in the study from the healthy elderly population >18 years old were included in the study.

Celiac disease was diagnosed according to the American College of Gastroenterology guidelines for celiac disease. All patients had tissue transglutaminase IgA and/or IgG antibody positives, and duodenal biopsies were

Marsh 1, 2, or 3 according to the modified Marsh classification (6).

The celiac patient group and groups formed from first-degree relatives were collected by retrospective file screening. Patients, first-degree relatives, and healthy control group, after being informed about the study and signing an informed consent, were divided into the patient group, first-degree relative group, and healthy control group. Inclusion criteria were adult age group >18 years old and consent to participate in the study by signing the informed consent. Individuals who did not provide consent to participate were excluded from the study. First-degree relatives who were diagnosed with celiac disease during the study were also excluded.

All groups were questioned for age, sex, anemia, osteoporosis, and diarrhea. Anti-tissue transglutaminase, anti-endomysium, and anti-gliadin antibodies were also recorded in the celiac patient group. Anemia was considered as hemoglobin <12 g/dL in women and hemoglobin <13 g/dL in men according to the World Health Organization (7). Osteoporosis was considered to be below -2.5 standard deviations of the total T score of the lumbar vertebrae (L1-L4) in the bone mineral densitometry of the patients (8).

A total of 285 (94 in the celiac patient group, 89 in the first-degree celiac group, and 102 in the healthy adult group) individuals were included in the study.

#### **Collection and evaluation of samples**

Peripheral blood samples were used as study material for the patient, first-degree relative, and healthy adult groups. All samples were delivered to the hematology laboratory in a purple-capped hemogram tube. DNA isolations of the samples were maintained at -20 °C until the working phase using the Arrow DNA Isolator (Nor-Diag ASA, Frysjaveien, Oslo, Norway). The study was performed by the Sequence-Specific Oligonucleotide (SSO) method, and the LAB Type™ SSO Class II DQA1/DQB1 Typing Test (One Lambda; Thermo Fisher Scientific, Canoga Park, CA, USA) was used as the test kit. Samples were centrifuged at 4100 rpm for 5 min as standard (device core is NF 400). Polymerase chain reaction was performed using the Kyrathec Super Cycler Trinity (Kyrathec, Mansfield, Queensland, Australia). Test analysis was performed using the Luminex Lab Scan 100™ Xmap instrument (Luminex Corp., Austin, TX, USA), and data analysis was performed using the same HLA Fusion V.356 computer program.

**Statistical analysis**

Data sets in the qualitative structure were expressed in number (n) and percentage (%). Chi-square tests were applied to variables in the categorical structure. The two proportions Z test was used in the intercell comparisons of the chi-square tables. A p value <0.05 was considered significant. All data analyses were performed using the Statistical Package for Social Sciences version 22.0 package programs (IBM Corp.; Armonk, NY, USA).

Written informed consent was obtained from patients who participated in the study.

Ethics committee approval was obtained from the ethics committee of Eskişehir Osmangazi University School of Medicine (approval no. 23.02.2016-80558721/31).

**RESULTS**

The total number of cases in the celiac disease group in the study was 94 (64 women and 30 men).

HLA-DQ2/DQ8 positive patients were 69. The rate was 73.4%. The total number of cases in the first-degree relative group with celiac disease was 89 (35 men and 54 women). The number of HLA-DQ2/DQ8 positive cases was 46. The rate was 51.7%. The total number of healthy adult cases is 102 (68 women and 34 men). HLA-DQ2/DQ8 positive cases were 29. The rate was 28.4%.

Table 1 shows the HLA-DQ2/DQ8 tissue group presence rates in all groups. There was a significant relationship between HLA-DQ2/DQ8 presence in all groups.

Table 2 shows the distribution of HLA-DQ2/DQ8 tissue groups in all groups. The distribution of HLA-DQ2/DQ8 in all groups was different (p=0.000).

In the celiac patient group, there were no significant differences between the HLA tissue groups in diarrhea (p=0.087), osteoporosis (p=0.215), anemia (p=1.000), tissue transglutaminase antibodies (p=0.295), anti-gliadin antibodies (p=0.104), anti-endomysium antibodies (p=0.243).

**Table 1.** HLA-DQ2/DQ8 tissue group presence rates in patients with celiac disease, first-degree relatives, and healthy adult groups

Groups		HLA-DQ2/DQ8		Total	p
		-	+		
Patients		25	69	94	0.000
		26.6%	73.4%	100.0%	
	First-degree relatives	43	46	89	
	48.3%	51.7%	100.0%		
Healthy groups		73	29	102	
		71.6%	28.4%	100.0%	
Total		141	144	285	
		49.5%	50.5%	100.0%	

**Table 2.** HLA-DQ2/DQ8 tissue group distribution in patients with celiac disease, first-degree relatives, and healthy adult groups

Groups		HLA tissue type			p
		-	DQ2	DQ8	
Patients		25	65	4	0.000
		26.6%	69.1%	4.3%	
	First-degree relatives	43	46	0	
	48.3%	51.7%	0.0%		
Healthy groups		73	29	0	
		71.6%	28.4%	0.0%	
Total		141	140	4	
		49.5%	49.1%	1.4%	

## DISCUSSION

Although duodenal biopsy is mandatory for the diagnosis of celiac disease according to the current guidelines, it has recently been argued that a diagnosis of celiac disease can be made without biopsy. Some investigators have suggested that duodenal biopsy may not be performed when there is a high level of tissue transglutaminase antibody (9-12).

Sometimes, these tests are inadequate in certain cases in the definite diagnosis of this disease. In the vast majority of patients, HLA-DQ2 has less HLA-DQ8 gene expression. It is stated that the screening of the HLA tissue group may be performed in close relatives of patients with celiac disease who want risk identification. It is also thought that HLA-DQ2 and HLA-DQ8 negativity are excluded in cases with suspicious histological and serological diagnoses (6). According to the European Pediatric Gastroenterology-Hepatology and Nutrition Society guidelines, although there are no data on this issue since there are not enough data on adult patients, despite the fact that tissue analysis is used to diagnose the disease in the pediatric age group and to scan first-degree relatives, tissue analysis is not used. We wanted to do a research on this area. In our study, we investigated the presence of HLA-DQ2/DQ8 in adult patients with celiac disease aged  $\geq 18$  years, first-degree relatives of patients with celiac disease, and healthy adults and found that HLA-DQ2/DQ8 positivity was significantly different in patients, first-degree relatives, and healthy adults. The distribution of HLA-DQ2/DQ8 among the groups was also different from the highest to the lowest in the form of the celiac disease group, first-degree relative group, and healthy adult group and was statistically significant.

There are many studies worldwide of HLA tissue analysis related to disease diagnosis and clinical status. In a study involving the pediatric population in Turkey, the positivity rates were 76% for HLA-DQ2 and/or HLA-DQ8, 67% for HLA-DQ2, and 25% for HLA-DQ8 in patients with celiac disease. Among them, 24% were HLA-DQ2 and HLA-DQ8 negative. The incidence of HLA-DQ2 in the control group was 18.8%. HLA-DQ8 incidence was 5.7% in the control group (13). In our study, HLA-DQ2/DQ8 positivity rate was 73.4%. HLA-DQ2 positivity rate was 69.1%. HLA-DQ8 positivity rate was 4.3%. Among them, 26.6% were HLA-DQ2 and HLA-DQ8 negative. In the healthy group, HLA-DQ2 positivity rate was 28.4%. HLA-DQ8 positivity rate was 0%. We also worked with first-degree relatives. HLA-DQ2 positivity rate was 51.7%, and HLA-DQ8 positivity rate was 0% in them. The results of these two studies

in Turkey are almost similar. On the other hand, we found a lower HLA-DQ8 positivity. In a study very similar to ours in Jordan, the HLA-DQ2 ratio was 80% in patients, 66% in first-degree relatives, and 32% in the control group (14). In a study in Iran, the HLA-DQ2/DQ8 positivity rates were 97% and 58% in the control group. The HLA-DQ8 ratio is 25.4% (15). In a study in Libya, the prevalence of HLA-DQ2 and HLA-DQ8 genes in the general population was found to be higher than in Italy (16). In a study on Bedouin, the HLA-DQ2 and HLA-DQ8 high-risk genotypes were found similar to those observed in Northern and Southern Europeans in Bedouin's celiac disease (17). In a Chilean study, it has been shown that celiac disease is primarily due to DQ8 conformation in patients (18).

Some researchers have investigated the relationship between the clinic and HLA status of the disease. In another study in Iran, HLA-DQ2 was associated with the severity of the disease, whereas HLA-DQ8 was observed to be more mild in the presence, and it was suggested that first-degree relatives of patients with celiac disease should be screened for HLA-DQ2 and HLA-DQ8 for possible early diagnosis and treatment (19). In a study comparing New Yorkese and Parisian patients, HLA-DQ8 was found more in New York patients, and HLA-DQ2 was found more in Parisian patients, but there was no link between tissue group and disease severity (20). Similarly, in our study, we did not find any association between clinical signs and HLA haplotypes. The relationship between the HLA haplotype and the clinic is not clear.

Sumník et al. (21) found that the HLA-DQ2 tissue group is more in children with type 1 diabetes mellitus and celiac disease than in children with diabetes without celiac disease. There was no difference for HLA-DQ8. They suggested that the celiac screening protocol in children with diabetes can be individualized according to DQ2 positivity.

Erriu et al. (22) found a link between oral manifestations and HLA-DQ2. Tümer et al. (23) investigated different patterns of HLA antigen in patients with celiac disease and found a high relative risk with HLA-B8, DR3, and DR3-DR4.

It is seen that the results of the association between HLA tissue group and clinical findings were different in many studies. HLA tissue groups at risk vary in different populations and different geographical regions. HLA-DQ2/DQ8 was absent in 26.6% of our patients. Different HLA tissue groups or susceptibility factors may have been active in this patient group.

In conclusion, to our knowledge, our study is the first study of HLA tissue analysis in an adult celiac patient group in a Turkish population. Clinical condition, autoantibodies, and histopathological findings are already available for diagnosis of the disease. In cases where these tests are inadequate, the presence of HLA-DQ2/DQ8 may be helpful in diagnosing celiac disease. However, the diagnosis of celiac disease cannot be excluded based on the results of HLA haplotyping in a Turkish population. HLA-DQ genotyping is not indicated in the initial evaluation of celiac disease. It can be used for the screening of first-degree relatives of patients with celiac disease. It is not appropriate to use it as a screening test in community screening since HLA-DQ2/DQ8 positivity is detected in a significant proportion in the healthy adult group. There is a need for more controlled studies to support our claims, especially in the Turkish society.

**Ethics Committee Approval:** Ethics committee approval was obtained from the ethics committee of Eskişehir Osmangazi University School of Medicine (Approval Number: 23.02.2016-80558721/31).

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

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