The incidence of HLA-DQ2/DQ8 in Turkish children with celiac disease and a comparison of the geographical distribution of HLA-DQ

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Abstract

Introduction: Celiac disease (CD) is an auto-immune enteropathy that occurs in genetically pre-disposed people as a result of the consumption of gluten-containing foods.

Aim: To identify the incidence of HLA-DQ2 and HLA-DQ8 observed in children with CD.

Material and methods: In this study, we focused on children ranging in age from 2 to 18 years and diagnosed with celiac disease. In our patients diagnosed with CD, in addition to tissue transglutaminase antibodies (anti-tTG), we also evaluated HLA-DQ2 B1 and HLA-DQ8 B1 alleles using the method of polymerase chain reaction (PCR)/sequence-specific oligonucleotide probes (Luminex®). The detection of 0201/0202 for HLA-DQ2 allele and 0302 for HLA-DQ8 allele was accepted as a positive result.

Results: The mean age of our patients with celiac disease was 7.42 ±3.18 years, and the female/male ratio was 1.5/1. Seventy-six percent of our patients were HLA-DQ2 and/or HLA-DQ8 positive, 67% were HLA-DQ2 positive, and 25% were HLA-DQ8 positive. Nevertheless, 24% of them were HLA-DQ2 and HLA-DQ8 negative. The incidence of HLA-DQ2 in the control group was 18.8% with a significant difference compared to the HLA-DQ2 incidence in the patient group (67%) ($p < 0.05$). Similarly the HLA-DQ8 incidence in the control group (5.7%) was significantly lower than the incidence in the patient group (25%) ($p < 0.05$).

Conclusions: The incidence of the patients diagnosed with CD, who are HLA-DQ2 and HLA-DQ8 negative, varies among different populations.

Introduction

Celiac disease (CD) is an auto-immune enteropathy that occurs in genetically pre-disposed people as a result of the consumption of gluten-containing foods. Gluten is a protein found in wheat and in other grains (e.g. barley, rye, oats). Celiac disease is observed in both children and adults and is a lifelong disorder [1].

Major histocompatibility complex (MHC) is a gene found in all vertebrates, and it has certain functions related to the immunity in some of them. The human version of MHC is HLA [2]. In humans, the gene region encoding MHC antigens is a sequential DNA locus close to the centromere on the short arm of chromosome 6 [3]. HLA antigens are categorised into three groups as class I, class II, and class III according to their structures and functions [4]. Regarding the celiac disease, the basic determinants of the genetic susceptibility for celiac disease are the MHC class II HLA-DQA and DQB genes (these genes are encoded by the histocompatibility region on the short arm of chromosome 6) [5].

The role of tissue groups in the diagnosis of celiac disease gains increasing importance. HLA-DQ2 and/or HLA-DQ8 have low specificity for CD (54% on average), and their value for the prediction of celiac disease is low. On the other hand, studies have shown that the susceptibility of HLA-DQ2 and DQ8 in CD is high (96.2% on average) and the possibility of celiac disease in people who are negative for both of these alleles is extremely low [6, 7].

The golden standard in the diagnosis of celiac disease is small bowel biopsy. Currently, small bowel biopsy is recommended for cases with positive serology or with negative serology but strong clinical suspicion [8]. Typical findings in celiac disease are increased intraepi-

thelial lymphocytes, crypt hyperplasia, and total villous atrophy (also called "flat mucosa") [9].

Presently there is no consensus on diagnosing CD without carrying out small bowel biopsy. The last version of ESPGHAN's guideline states that patients with apparent clinical symptoms and with highly positive titration of anti-tTG IgA (10× higher than normal) with positive endomysial antibodies (EMA) and with positive HLA-DQ2 and/or HLA-DQ8 heterodimer can be diagnosed without a biopsy after negotiation with the patient's family. However, in patients with low titration of anti-tTG antibodies and negative EMA test, biopsy is definitely necessary. In patients diagnosed without biopsy, significant relief of symptoms and normalisation in CD antibodies should be closely monitored [8].

Regarding these data, analysis of HLA-DQ2 and HLA-DQ8 haplotypes in celiac disease is essential for the diagnosis and prevention of misdiagnosis. Although there are some studies focused on this subject in the literature, further studies are needed.

Aim

The aim of our study was to identify the incidence of HLA-DQ2 and HLA-DQ8 tissue groups in the patients diagnosed with CD.

Material and methods

In this study, we focused on children ranging in age from 2 to 18 years, diagnosed with celiac disease in the Akdeniz University Paediatric Gastroenterology Outpatient Clinic between September 2010 and May 2015. We obtained the approval of the Local Ethics Committee for the clinical trials of Akdeniz University before the study startup (Date: June 10, 2015, Approval Number: 278). In the Paediatric Gastroenterology Outpatient Clinic of our hospital, for diagnostic purposes, we evaluated anti-tTG IgA, anti-tTG IgG antibodies, and HLA-DQ2 and HLA-DQ8 alleles with the "polymerase chain reaction (PCR)/sequence-specific oligonucleotide probes" method (Luminex®) in patients with a suspicion of CD. For a definitive diagnosis, the histopathological analysis of multiple duodenal biopsies was done. Regarding the results of the histopathological analysis, intraepithelial lymphocytosis is classified as Marsh 1, a moderate decrease of the ratio of villus length/crypt depth as Marsh 2, partial villous atrophy as Marsh 3a, subtotal villous atrophy as Marsh 3b, and total villous atrophy as Marsh 3c [8]. In patients diagnosed with celiac disease, the obtained HLA-DQ2 and HLA-DQ8 allele results are retrospectively evaluated and negative and positive results are determined. During the interpretation of the HLA-DQ2 and HLA-DQ8 allele levels, the detection of 0201 and 0202 for HLA-DQ2 B1 and the detection of 0302 for HLA-DQ8 B1 are regarded as positive [10]. As a control group for HLA-DQ2, we evaluated the data of a postgraduate thesis focused on the HLA antigen levels of 771 subjects in the Immunology Department of the Central Laboratory in the Balcalı Hospital of the Medical Faculty of the Çukurova University [11]. As a control group for HLA-DQ8, we evaluated the data of a similar postgraduate thesis focused on the HLA antigen levels of 910 subjects in the Immunology Department of the Central Laboratory in the Medical Faculty of the Çukurova University [12]. We compared the HLA-DQ2 B1 and HLA-DQ8 B1 results of our patients diagnosed with CD with the HLA-DQ2 [11] and HLA-DQ8 [12] results of the healthy populations and assessed the incidence and significance of the HLA-DQ2 B1 and HLA-DQ8 B1 alleles in our patients diagnosed with celiac disease.

Statistical analysis

We used the software SPPS ver. 15.0 (SPSS Inc., Chicago, IL, USA). The comparative analysis of the data was performed with χ^2 test and Fisher's Exact test. For the descriptive statistical analysis of the data, we used average ± standard deviation for the numeric variables and numbers and percentages for the categorical variables. The accepted significance level was $p < 0.05$.

Results

One hundred and twenty-nine patients diagnosed with celiac disease were enrolled into our study. Twenty-seven of these patients were not included in the evaluation because their HLA-DQ2 B1 and HLA-DQ8 B1 alleles were not investigated, but they had positive anti-tTG antibodies and they were diagnosed with CD. The remaining 102 patients were included in the study, as their diagnosis was confirmed with the clinical, laboratory, and histopathological findings. The mean age of our patients with celiac disease was 7.42 ±3.18 years and the female/male ratio was 1.5/1. In our study, the most frequently observed complaints were diarrhoea (83%), abdominal pain (40%), growth retardation (38%), and abdominal distension (41%). Analysis of the histopathological classification showed that 68.9% of our patients had Marsh 3, 2.4% had Marsh 2, and 9.7% had Marsh 1. The analysis of our patients with CD according to the tissue groups revealed that 76% of them were HLA-DQ2 and HLA-DQ8 positive, 67% of them were HLA-DQ2 positive, and 25% were HLA-DQ8 positive. However, 24% of our patients were neither HLA-DQ2 nor HLA-DQ8 positive. The comparative analysis with the control group [11] showed that the incidence of HLA-DQ2 in the control group was 18.8%, but it was 67% in the patient group. The difference between these groups was signifi-

cant ($p < 0.05$). A similar significance was also observed for the incidence of HLA-DQ8 between the control group [12] (57%) and the patient group (25%) ($p < 0.05$). The complaints during the outpatient visits, average ages, and distribution according to the histopathological classification of patients with HLA-DQ2, HLA-DQ8, HLA-DQ2, and/or HLA-DQ8 and without HLA-DQ2 and HLA-DQ8 and their comparison is listed in Table I.

The comparative evaluation of our patients with CD regarding the HLA-DQ2, HLA-DQ8, HLA-DQ2, and/or HLA-DQ8 did not show any significant difference. On the other hand, we observed that complaints of the patients without HLA-DQ2 and HLA-DQ8 were less frequent (Figure 1).
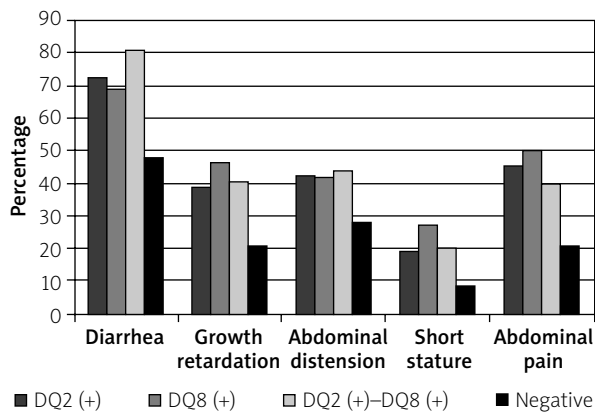
Discussion

There are numerous studies in the literature focused on the incidence of HLA-DQ in patients diagnosed with celiac disease. In the majority of these studies HLA-DQ was evaluated, and in some studies only HLA-DQ A1 or B1 alleles were evaluated.

One of the HLA-DQ studies was conducted by Karell *et al.* [6] In this study, which was done in France, Italy, Finland, Norway, and England, the incidences were between 87% and 93.7% for HLA-DQ2, between 5% and 8% for HLA-DQ8, and between 89.4% and 96.7% for HLA-DQ2 and/or HLA-DQ8. In the study of Vidales *et al.* [13], the determined incidences were 93.4% for HLA-DQ2; 2.4% for HLA-DQ8, and 95.6% for HLA-DQ2 and/or HLA-DQ8. The incidences of another study by

Figure 1. The distribution of celiac groups regarding the complaints of the patients during their outpatient visits

Table I. The distribution of the celiac patients and groups regarding the demographic characteristics, complaints during the visits, and histopathological classifications

Parameter	DQ2 (+)	DQ8 (+)	DQ2 and/or DQ8 (+)	DQ2 and DQ8 (–)	Total
Age, average	6.97 ±3.32	7.12 ±2.78	7.08 ±2.94	6.85 ±3.24	7.42 ±3.18
Gender (M/F)	43/25	15/11	44/31	15/10	65/37
Complaints during visits (%):					
Diarrhoea	73	69	81	48	83
Growth retardation	40	46	40	20	38
Abdominal distension	43	42	43	28	41
Short stature	19	27	19	8	18
Chronic abdominal pain	46	50	40	20	40
Delayed puberty	1	4	1	0	1
Iron deficiency anaemia	2	4	2	0	2
Constipation	0	0	0	0	0
Hypertransaminasaemia	2	0	0	0	1
Recurrent oral aphthae	0	0	0	0	0
Histopathological classification (%):					
Marsh 1	5	9	8	11	9.7
Marsh 2	21	18	23	21	21.4
Marsh 3	74	62	65	66	68.9

Table II. Studies related with HLA-DQ2 and/or HLA-DQ8 in patients with celiac disease

Author, year (reference no.)	Country	Patient (P)/ Control (C)	HLA-DQ2 (+) P/C (%)	HLA-DQ8 (+) P/C (%)	HLA-DQ2 and/or DQ8 (+) P/C (%)
Djilali-Saiah, 1998 [19]	France	101/130 *DQ B1* *DQ A1B1*	68/43 70.2/34.6		
Pérez-Bravo, 1999 [27]	Chile	62/124 *DQ A1B1*	11.3/	25.8/	37.1/
Sumnik, 2000 [28]	Czechoslovakia	15/186 *DQ A1B1*	80/49	67/66	100/
Tümer, 2000 [18]	Turkey	33/77 *DQ A1B1*	52/34		
Neuhausen, 2002 [29]	Israel	23/ *DQ A1B1*	82.6/	56.5/	100/
Kaur, 2002 [20]	India	35/135 *DQ A1B1* *DQ B1*	97.1/ 100/		
Karell, 2003 [6]	France	92/ *DQ A1B1*	87/	6.5/	93.5/
	Italy	302/ *DQ A1B1*	93.7/	5.6/	89.4/
	Finland	100/ *DQ A1B1*	91/	5/	96/
	Norway	326/ *DQ A1B1*	91.4/	5.2/	96.6/
	England	188/ *DQ A1B1*	87.8/	8/	96.7/
Johnson, 2004 [30]	New York/Paris	44/66 *DQ A1B1*	86/93	41/21	
Vidales, 2004 [13]	Spain	136/ *DQ A1B1*	93.4/	2.1/	95.6/
Kuloğlu, 2008 [17]	Turkey	75/100 *DQ A1B1*	84.7/27		
El-Akawi, 2010 [31]	Jordan	44/53 *DQ A1B1*	80/32		
Piccini, 2012 [14]	Italy	89/70 *DQ A1B1*	64/21.3	16.8/10	77.5/30
Erriu, 2013 [21]	Italy	44/ *DQ B1*	79.5/		
Rostami-Nejad, 2014 [26]	Iran	59/151 *DQ A1B1*	83.03/35.09	25.4/	97/58
Pallav, 2014 [15]	USA	72/44 *DQ A1B1*	69.5/	25.3/	95.5/30
Delgado, 2014 [16]	Spain	91/158 *DQ A1B1*	96.7/63.3	4.4/15.2	100/74
Zamani, 2014 [22]	Iran	120/100 *DQ B1*	75/	14/	98/44
Our study, 2015	Turkey	102/ *DQ2-771* *DQ8-910* *DQ B1*	67/18.8	25/5.7	76/

Piccini *et al.* [14] were 64% for HLA-DQ2, 16.8% for HLA-DQ8, and 77.5% for HLA-DQ2 and/or HLA-DQ8.

Pallav *et al.* [15] obtained similar results regarding the incidences: 69.5% for HLA-DQ2, 25.3% for HLA-DQ8, and 95.5% for HLA-DQ2 and/or HLA-DQ8.

Concurrently in a study, Delgado *et al.* [16] found the following incidences: 96.7% for HLA-DQ2, 4.4% for HLA-DQ8, and 100% for HLA-DQ2 and/or HLA-DQ8.

In some of the studies focused on patients with celiac disease and conducted in our country, Kuloğlu *et al.* [17] reported the incidence of HLA-DQ2 as 84.7%; Tümer *et al.* [18] reported the incidence of HLA-DQ2 as 52%.

Among the studies on HLA-DQ and HLA-DQ B1, Djilali-Saiah *et al.* [19] found that 70.2% of the patients with CD had HLA-DQ2, and 68% had HLA-DQ2 B1. In another study Kaur *et al.* [20] showed that 97.1% of the patients diagnosed with CD had HLA-DQ2 and 100% of them had HLA-DQ2 B1.

Among the studies evaluating only HLA-DQ B1, Erriu *et al.* [21] reported that 79.5% of the patients diagnosed with CD and Zamani *et al.* [22] reported that 75% of the patients diagnosed with CD had HLA-DQ2, 14% had HLA-DQ8, and 98% had HLA-DQ2 and/or HLA-DQ8.

The comparison of all these studies reveals that the results of the studies evaluating HLA-DQ haplotype and HLA-DQ B1 allele are similar. In our study, the results regarding the incidence of HLA-DQ2 was 67% and the incidence of HLA-DQ2 and/or HLA-DQ8 was 76%, and they were in line with the results reported in the literature.

Surprisingly, the incidence of HLA-DQ8 in our study (25%) was much higher than the studies in the literature reporting lower incidences (Cameroon 0.6%; Italy 2%; Hungary 2.3%; USA 4.2%; Finland 6.4%; Japan 7.6%) [23, 24]. However, our result is in line with the result of the study conducted on HLA-DQ8 by Catassi *et al.* [25] in 2009 on Turkish people (22%), North American natives (25.3%), Mexicans (28.3%), and Bushmen (an indigenous group of southern Africa) (30%). In addition to that, our results also confirm the results of Rostami-Nejad *et al.* [26] on Iranian people (25.4%) and the results of Pérez-Brave *et al.* [27] in Chile (25.8%).

There are some published studies in the literature reporting higher incidences of HLA-DQ8 than the incidences of our study. In the study conducted by Sumnik *et al.* [28] in Czechoslovakia in 2000, the incidence of HLA-DQ8 was 67% and in the study conducted by Neuhausen *et al.* [29] in Israel with Bedevins the incidence of HLA-DQ8 was 56.5%.

All these results confirm that the incidence of HLA-DQ8 varies in different geographical regions.

According to the data originating from western countries [4, 13, 15, 16, 28], the percentage of patients with celiac disease that are HLA-DQ2 and HLA-DQ8 negative is between 0 and 10%, but there are also other studies reporting higher percentages. In their study conducted in Chile, Pérez-Bravo *et al.* [27] reported that 62.9% of patients were HLA-DQ2 and HLA-DQ8 negative. The result reported by Piccini *et al.* [14] and obtained in Italy (22.5%) is in line with our result (24%). These results show that the incidence of patients diagnosed with celiac disease and who are HLA-DQ2 and HLA-DQ8 negative varies among different populations.

The HLA DQ results of the patients with celiac disease in our study and in other studies are shown in Table II.

Conclusions

The incidence of the patients diagnosed with CD, who are HLA-DQ2 and HLA-DQ8 negative, varies among different populations. Therefore, it is believed that inherited celiac disease, besides HLA-DQ, may also be closely related to other tissue groups and non-HLA genes with an increasing importance recognised in recent years.

Conflict of interest

The authors declare no conflict of interest.

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