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HUMAN LEUKOCYTE ANTIGENS IN TURKISH PEDIATRIC CELIAC PATIENTS*

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With the aim to determine the frequency of human leukocyte antigen phenotypes of celiac disease in Turkey, thirty celiac patients fulfilling the European Society of Pediatric Gastroenterology and Nutrition criteria were included in the study. The mean age of the study population was 5.8 ± 4.3 years and of the control subjects was 32.6 ± 6.7 years.

The human leukocyte antigens -A, -B, -DR and -DQ were studied serologically by micro lymphocytotoxic reaction.

It was found that human leukocyte antigens A-25(10), -B8, -DR18(3) and -DQ2 were more significantly frequent in the celiac population than in the control group. Children with antigen -B8 showed a five times higher risk for celiac disease and those with antigen -DQ2 showed a nine times higher risk. It was determined that human leukocyte antigen -B4 had a protective role in celiac disease.

The study suggests that the human leukocyte antigen -A25(10) is a phenotype particularly encountered in Turkish pediatric celiac patients. *Key words: human leukocyte antigen HLA types, celiac disease, Turkish children.*

Celiac disease (CD), an enteropathy caused by gluten-containing foods, is the most common cause of malabsorption in infants and children[1].

Genetic, environmental and immune system factors may play an important role in the pathogenesis of the disease[1]. It was reported for the first time in 1972 that relationship exists between celiac disease and human leukocyte antigen (HLA)-B8, and in 1983 between CD and HLA-DQ2 group[2,3]. The association with DR3 and DR7 is explained by the linkage disequilibrium of these alleles with the DQ2 allele[3]. Furthermore, HLA typing by molecular method showed a strong association of CD with the DQA1*0501 and dDQB1*0201 alleles combination at the DQ subregions[4]. The frequency of HLA subtypes in the ethnic populations varies significantly. In northern Europe HLA-DR3 was the most common antigen among celiac patients; in Spain and Italy, HLA-DR3 and -DR7 were the most common[5-7].

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In view of this, we studied the frequency of HLA types in Turkish pediatric celiac patients. To our knowledge, is the first such study.

Material and Methods

Thirty celiac patients fulfilling the European Society of Pediatric Gastroenterology and Nutrition criteria[8] were included in our study. After the establishment of the diagnosis by clinical presentation and hyperplastic villous atrophy in the first biopsy, patients recovered on a gluten-free diet. The mean age of the study population was 5.8 ± 4.3 (20 female, 10 male). Healthy control subjects were 30 randomly selected blood donors for renal transplant recipients. The mean age of the control subjects was 32.6 ± 6.7 years (15 female, 15 male).

The HLA-A, -B, -DR and -DQ were studied serologically by the standard microlymphocytotoxic method. Lymphocyte preparation was obtained from heparinized peripheral blood by ficoll[9]. These lymphocytes were purified by MN-Kwik (Lymphokwik MN cell/one lambda America). The remaining T and B lymphocytes were added to the HLA-ABC tray for determination of class I HLA.

For HLA class II typing, B lymphocytes were first separated from T lymphocytes using B1 and B2 kwik (lymphokwik B1 cell and lymphokwik B2 cell/one lambda America), then added to HLA class II tissue typing trays. The trays were scored using inverted light microscope[10].

The statistical significance of differences in the frequency of HLA types between celiac patients and controls was calculated by chi-square analysis. When the number of groups was below five, Fisher's exact test was utilized. In order to estimate relative risk, the odds ratio method was also used. The parents of the subjects were invited to give their consent after the purpose and requirements of the study were explained to them.

Results

The distribution of HLA in celiac patients and controls is shown in Tables I and II.

In celiac patients, HLA-A2 (43.3%), HLA-B6 (56.5%), HLA-DR53 (83.3%), and HLA-DQ7(3) (43.3%) were the most common HLA types observed in HLA groups -A, -B, -DR and -DQ respectively (Tables III and IV).

In the control group, HLA-A2 (46.6%), HLA-B4 (93.3%), HLA-DR53 (76.6%), and HLA-DQ4 (53.3%) were the most common HLA types obtained in HLA groups -A, -B, -DR and -DQ respectively (Tables III IV).

A significant positive association between CD and HLA-A25(10), -B8, -DR18(3) and -DQ2 ($p = 0.02$; $p = 0.01$; $p = 0.02$; $p = 0.002$, respectively) is evident

(Table V). But after the odds ratio test, only HLA-B8 and HLA-DQ2 demonstrated higher risk for celiac disease (five and nine times, respectively).

HLA-B4, -B7, -DR11(5), and -DQ6 antigens were significantly frequent in the control group (Table V). However, only HLA-B4 demonstrated a higher protective role (five times) (odds ratio: 0.5, interval: 0.01-0.31).

Table I: Distribution of HLA Types of the CD Patients

Name	HLA-AB	HLA-DR	HLA-DQ
S.Y	A3, A10, B6, B8	DR4, DR10, DR52, DR53	DQ2, DQ7(3)
G.Ç	A1, A2, B4, B13, B52(5)	DR7, DR11(5), DR52, DR53	DQ4, DQ7(3)
D.K.	A1, A9, B6, B8 B73	DR8, DR9, DR52, DR53	DQ4, DQ6(1)
Ş.K.	A25(10), B6, B8, B65(14)	DR7, DR15(2), DR52, DR53	DQ4
M.Y	A2, A25(10), B4, B6, B35, B51(5)	DR10, DR15(2), DR52, DR53	DQ2, DQ4
N.Ç	A1, A24(9), B4, B6, B8, B35	DR13(6), DR18(3), DR52	DQ2, DQ6(1)
S.K	A2, A11, B4, B6, B27, B35	DR1,DR4, DR52, DR53	DQ1, DQ3
N.B	A1, A19, B4, B17, B37	DR1, DR11(5), DR52, DR53	DQ1, DQ4
D.S	A2, A11, B4, B6, B40, B51(5)	DR8, DR14(6), DR52, DR53	DQ3, DQ4
Ş.E	A10, A11, B4, B6, B8, B44(12)	DR18(3), DR52	DQ6(1)
Z.O	A11, A24(9), B6, B8, B40	DR15(2), DR18(3), DR52, DR53	DQ1, DQ2
N.G	A1, A11, B6, B8, B18	DR7, DR11(5), DR52, DR53	DQ2, DQ4
G.A	A2, A3, B6, B22	DR1, DR12(5), DR52, DR53	DQ4, DQ7
H.Ş	A2, A66, B4, B27, B51(5)	DR4, DR15(2), DR52, DR53	DQ2, DQ7(3)
F.U	A1, A26(10), B4, B6, B35, B62(15)	DR11(5), DR13(6), DR52	DQ2, DQ7
U.B	A2, A24(9), B6, B8, B35	DR1, DR11(5), DR52, DR53	DQ7(3)
T.S	A1, A25(10), B6, B8	DR8, DR14(6), DR52	DQ1, DQ7(3)
M.Ö	A28, A36, B4, B6, B8, B12	DR4, DR18(3), DR52, DR53	DQ7(3)
E.M	A2, A3, B4, B6, B44(12), B51(5)	DR8, DR11(5), DR52, DR53	DQ6(1), DQ7(3)
D.E	A19, A28, B4, B6, B51(5), B78	DR1, DR11(5), DR52, DR53	DQ1, DQ2
A.K	A2, A30(19), B4, B51(5)	DR11(5), DR14(6), DR52	DQ6(1), DQ7(3)
C.K	A1, A3, B13, B35	DR7, DR15(2), DR52, DR53	DQ2, DQ7(3)
A.T	A2, A3, B14, B17	DR7, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
Y.G	A2, A32(19), B5, B16	DR14(6), DR18(3), DR52, DR53	DQ4, DQ6(1)
K.E	A2, A10, B27, B60(40)	DR8, DR11(5), DR52, DR53	DQ6(1), DQ7(3)
C.G	A3, A25(10), B22 B27	DR10, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
Z.Ç	A19, A24(9), B13, B51(5)	DR7, DR11(5), DR52, DR53	DQ3, DQ4
B.Y	A2, A25(10), B13, B60(40)	DR11(5), DR18(3), DR52, DR53	DQ2, DQ7(3)
M.S	A10, A11, B44(12), B62(15)	DR7, DR18(3), DR52, DR53	DQ2, DQ6(1)
E.Y	A9, A24, B8, B15, B62	DR3, DR4, DR52, DR53	DQ2

Table II: Distribution of HLA Types of the Control Group

Name	HLA-AB	HLA-DR	HLA-DQ
H.T.	A2, A3, B4, B6, B7, B44(12)	DR11(5), DR13(6), DR52	DQ3, DQ4
A.G	A1, A2, B4, B6, B7, B44(12)	DR4, DR11(5), DR52, DR53	DQ6(1), DQ7
D.G	A2, A3, B4, B13, B37	DR7, DR11(5), DR52, DR53	DQ1, DQ6
H.S	A3, A24(9), B4, B6, B17, B35	DR13(6), DR15(2), DR52	DQ1, DQ4
P.B	A2, B4, B6, B27, B35	DR10, DR13(6), DR52, DR53	DQ1, DQ3
A.S	A2, A24(9), B4, B6, B35, B44(12)	DR10, DR11(5), DR52, DR53	DQ4, DQ7
B.G	A2, A11, B4, B6, B51(5), B60(40)	DR11(5), DR13(6), DR52	DQ3, DQ7
İ.M	A10, A33(19), B6, B35	DR11(5), DR15(2), DR52, DR53	DQ1, DQ6
M.K	A2, A3, B4, B6, B7, B44(12)	DR8, DR11(5), DR52	DQ3, DQ6
H.A	A2, A19, B4, B27, B44(12)	DR10, DR11(5), DR52, DR53	DQ1, DQ3
F.B	A9, A11, B4, B6, B13, B35	DR11(5), DR13(6), DR52	DQ7(3), DQ4
H.T	A2, A19, B4, B27, B37	DR10, DR14(6), DR52, DR53	DQ6, DQ7
Ş.A	A2, A28, B4, B6, B44(12)	DR1, DR8, DR52	DQ1, DQ4
İ.T	A1, A30(19), B4, B5, B6, B7	DR11(5), DR15(2), DR52, DR53	DQ4, DQ6(1)
Z.S	A2, A24(9), B4, B6, B51(5), B60(40)	DR11(5), DR18(3), DR52	DQ4, DQ7(3)
B.Ş	A10, A11, B4, B6, B22, B44(12)	DR4, DR8, DR52, DR53	DQ6(1), DQ7(3)
M.G	A2, A24(9), B4, B6, B8 B44(12)	DR8, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
F.A	A3, A19, B4, B13, B51(5)	DR7, DR11(5), DR52, DR53	DQ3, DQ4
Y.D	A10, A28, B4, B6, B13, B38(16)	DR10, DR11(5), DR52, DR53	DQ1, DQ4
A.P	A3, A23(9), B4, B37, B51(5)	DR11(5), DR15(2), DR52, DR53	DQ4, DQ7(3),
M.A	A3, A32(19), B4, B17, B51(5)	DR4, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
M.K	A2, A10, B4, B51(5)	DR10, DR11(5), DR52, DR53	DQ4, DQ7(3)
Ü.K	A10, A30(19), B4, B17, B51(5)	DR10, DR14(6), DR52, DR53	DQ4, DQ3(7)
S.G	A24(9), A28, B4, B27, B37	DR8, DR11(5), DR52, DR53	DQ1, DQ7(3)
Ö.A	A9, A26(10), B4, B6, B27, B35	DR9, DR11(5), DR52, DR53	DQ2, DQ4
A.K	A1, A3, B6, B8, B35	DR11(5), DR15(2), DR52, DR53	DQ6(1), DQ7(3)
B.G	A10, A24(9), B4, B6, B8, B37	DR8, DR10, DR52, DR53	DQ2, DQ4
İ.A	A1, A3, B4, B17, B51(5)	DR1, DR15(2), DR52, DR53	DQ4, DQ6(1)
F.K	A2, A24(19, B4, B13, B51(5)	DR9, DR11(5), DR52, DR53	DQ4, DQ7(3)
S.Ü	A10, A30(19), B4, B6, B21, B35	DR8, DR14(6), DR52, DR53	DQ6(19), DQ7(3)

Table III: Frequency of HLA-A, -B in the Patient and Control Groups

HLA-A	Patient (%)	Control (%)	HLA-B	Patient (%)	Control (%)
A1	26.6	13.3	B4	43.3	93.3
A2	43.3	46.6	B5	3.3	6.6
A3	20	30	B6	56.6	0
A9	6.6	6.6	B7	0	13.3
A10	13.3	26.6	B8	36.6	10
A11	20	6.6	B12	3.3	0
A19	10	10	B13	13.3	16.6
A23(9)	0	3.3	B14	3.3	0
A24(9)	13.3	20	B15	3.3	0
A24	3.3	0	B16	3.3	0
A25(10)	16.6	0	B17	6.6	13.3
A26(10)	3.3	3.3	B18	3.3	0
A28	6.6	10	B21	0	3.3
A29(19)	0	3.3	B22	6.6	3.3
A30(19)	3.3	10	B27	13.3	16.6
A32(19)	3.3	3.3	B35	20	26.6
A33(19)	0	3.3	B37	3.3	16.6
A36	3.3	0	B38(16)	0	3.3
A66	3.3	0	B40	6.6	0
			B44(12)	10	26.6
			B51(5)	23.3	30
			B52	3.3	0
			B60(40)	6.6	6.6
			B62	3.3	0
			B65(14)	3.3	0
			B73	3.3	0
			B78	3.3	0

Table IV: Frequency of HLA-DR, -DQ in the Patient and Control Groups

HLA-DR	Patient (%)	Control (%)	HLA-DQ	Patient (%)	Control (%)
DRI	16.6	33.3	DQ1	16.6	26.6
DR3	3.3	0	DQ2	40	6.6
DR4	16.6	10	DQ3	10	20
DR7	23.3	6.6	DQ3(7)	0	3.3
DR8	16.6	23.3	DQ4	33.3	53.3
DR9	3.3	6.6	DQ6	0	13.3
DR10	10	26.6	DQ6(1)	33.3	26.6
DR11(5)	36.6	63.3	DQ7	6.6	13.3
DR12(5)	3.3	0	DQ7(3)	43.3	33.3
DR13(6)	6.6	16.6			
DR14(6)	13.3	10			
DR15(2)	23.3	26.6			
DR18(3)	23.3	3.3			
DR52	100	100			
DR53	83.3	76.6			

Table V: HLA Groups Associated with CD

HLA	Patient Group n	Control Group n	p	Odds Ratio*
B8	11	3	0.01	1.12-27.5
DR18(3)	7	1	0.02	0.96-204.9
DQ2	12	2	0.002	1.65-68.78
A25(10)	5	0	0.02	0.58-140.35
B4	13	28	0.00003	0.01-0.31
B7	0	4	0.03	0.01-2.39
DR11(5)	11	19	0.03	0.1-1.08
DQ6	0	4	0.03	0.01-2.39

*: Confidence interval: 95%.

Discussion

Human leukocyte antigen types have been used as a genetic marker of CD since 1972, and determining the frequency of specific HLA types in the population may suggest a predisposition to this disease[2].

Our results confirm a significant association of CD with HLA-A25(10), -B8, -DR18(3) and -DQ2. In different studies the frequency of HLA-B8 has been reported as 45-88 percent[11] in celiac patients. In the European Caucasian control group this ratio was 20 percent[12]. Our study result of 37 percent in celiac patients is similar to other reports.

In the present study, HLA-DQ2 was strongly associated with CD. Neverthless, the rate of DQ2+ patients was 40 percent, lower than reported in other series. The alleles DQA1*0501 and DQB1*0201, which encode for the HLA-DQ2 molecule, are found in over 90 percent of celiac patients from northern and southern Europe[13, 14]. At the same time, there is no increase in DQ1 and/or DQ8 (DR4+), which are expressed more in the -DQ2 population, as mentioned by Mantovani et al.[15] In the normal population, HLA-DQ is in strong linkage disequilibrium with HLA-DR3 and HLA-DR7 and, in view of this, it has been proposed that the significant association of CD with these latter antigens may be secondary to the association with the DQ2 antigen[16]. Recent studies have demonstrated that all gliadin specific T cells isolated from the small bowel only recognize gliadin antigen in the presence of HLA-DQ2 and not -DR and -DP class I molecules[17].

Because all reports about significant association between DR7 and CD concern pediatric patients, other works speculated that juvenile celiac patients are genetically different from adult celiac patients[7, 18, 19]. Our results are, however, not in keeping with this view, as we did not demonstrate an association between DR7 and CD. Meuli et al.[19] showed that there was a significant increase in the frequency of DR3 and DR7 in celiac patients.

The study of Betuel et al.[20], realized in adult patients, showed no difference between the HLA status of children and adults with CD, suggesting that the genetic heterogeneity appears to be more a result of different geographic origin than of patient's age. Thus, the strength of association is higher for DR3 and DQ2 in northern Europe, while DR7 is generally limited to southern Europe[14, 21].

The distribution of HLA in our population was between that of the Arabic and European populations. The absence of HLA-A1 and B8, which is very characteristic for these populations, did not exist in our group. In the Turkish population, HLA-A2, -DR2, -A9, -Cw4, -DR4, and -B35 were the most frequent antigens, and HLA-A29, -B39, -B15, -A23, and -A37 were the less frequent antigens. Thus, the antigens found to be in strong association with CD did not show a high frequency in the Turkish population. This observation suggests that the geographic difference alone may not be sufficient to explain the genetic heterogeneity of HLA distribution.

We could not find any report in the literature concerning the association of HLA-A25(10) and CD. This latter antigen may be a phenotype particularly encountered in Turkish pediatric celiac patients.

Our data indicate that HLA-B4 plays a protective role against the development of CD. Because more genes are less frequent in celiac patients than in healthy subjects, this finding supports the hypothesis that it compensates for the increased frequency of the other genes[18].

In conclusion, our study supports the hypothesis previously put forward by others that DQ2, B8, DR3 (the term HLA-DR18(3) indicates that -DR18 has split off HLA-DR3) are the phenotypes more strongly associated with CD, and also demonstrates that HLA-A25(10) may be an indicator particularly for Turkish pediatric celiac patients. Although linkage studies between HLA types and CD are now realized at the genetic level using molecular biology technology, serological studies are still of great value when molecular biology is not available.

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