HLAs in children with minimal change disease and other types of nephrotic syndrome in the southern part of Turkey

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The aim of this study was to investigate the human leukocyte antigen (HLA) profile of children with nephrotic syndrome in the southern part of Turkey. Seventy-eight children with nephrotic syndrome were studied for the frequency of class I and class II human leukocyte antigens. Forty-seven of them were steroid sensitive nephrotic syndrome (minimal change disease-MCD) and 31 were other types of nephrotic syndrome. The results were compared with 133 healthy subjects for HLA groups. HLA B13, Cw5, Cw7, DR4, DR7, DRw10, Drw15(2) and DQ2 in the MCD group and HLA A31, B8, B13, B17, Cw2, Cw6, Cw7, DRw10 and DRw12 in the non-MCD group were found significantly increased when compared to healthy controls. MCD patients with frequent relapses had higher frequencies of both Cw6 and DR1 ($p < 0.005$) and MCD patients with infrequent relapses had a higher frequency of Cw7 ($p < 0.05$). In conclusion, HLA groups may help in the early diagnosis of these variants.

Key words: HLA, nephrotic syndrome, Turkish children.

Nephrotic syndrome (NS), one of the common renal diseases in childhood, is characterized by proteinuria, hypoalbuminemia, hypercholesterolemia, and edema. Minimal change disease (MCD) is the most frequent cause of childhood nephrotic syndrome, accounting for 80 to 85 percent of all cases. Other various subtypes of chronic glomerulonephritis (CGN) result in severe proteinuria and represent an important cause of nephrotic syndrome in children. Of these subtypes, focal segmental glomerulosclerosis (FSGS) is the most frequent one, followed by mesangial proliferation (MesPGN), membranous (MGN) and membranoproliferative glomerulonephritis (MPGN)[1]. The cause of idiopathic nephrotic syndrome remains unknown. Treatment of NS with "immunosuppresive" drugs suggested that the disease was mediated by an immunologic mechanism. Some diseases with immunologic bases are known to be associated with human leukocyte antigen (HLA) system[2]. Also, strong associations with certain HLAs in children with MCD have been shown in several studies. In this study, we investigated HLAs in patients from the southern part of Turkey with MCD and other types of nephrotic syndrome diagnosed by biopsy. We compared our findings in patients and controls to determine the associations with HLAs.

Material and Methods

Of all patients with NS followed at the Department of Pediatric Nephrology, Çukurova University, School of Medicine, 47 children with MCD (the criteria of the International Study of Kidney Disease in Children were used for the diagnosis) and 31 with non-MCD (7 MPGN, 2 FSGS, 19 MesPGN, 3 diffuse proliferative glomerulonephritis) diagnosed by biopsy were included in the study. All patients were Turkish children from the southern part of Turkey. In the MCD group, 37 were infrequent relapsers and 10 were frequent relapsers. The control population consisted of 133 living kidney donors. They were unrelated to each other and were healthy Turkish people from the southern part of Turkey.

HLA typing was performed using the standard microlymphocytotoxicity test[3]. Separated lymphocytes were used for HLA -A, -B and -C

typing by the microlymphocytotoxicity technique; some separated lymphocytes were treated with magnetic beads for separation of class II positive lymphocytes. Acridine orange/ethidium bromide staining of B cells was performed for HLA DR typing; fluorescence microscopy was used for this purpose. The significance of association was tested by Fisher's exact test or the chi-square test, as appropriate. All p values were corrected with the Benferroni correction. The relative risk (RR) was calculated by Odd's ratio. Ninety-five percent confidence intervals (95% CI) for relative risk were calculated by ANOVA test.

Results

A significant increase in HLA B13, Cw5, Cw7, DR4, DR7, DRw10, DRw15(2), and DQ2 and a significant decrease in HLA A24(9), Bw4, Bw6, DR2, and DR5 were found in the MCD group when compared with the control healthy group. HLA-B17 was found in 17 percent of the patients in the non-MCD group, but in none of the MCD patients ($p < 0.005$). Seven of the 31 non-MCD patients were positive for HLA DRw12 while none of the MCD patients was positive, but the difference was not statistically significant. In the non-MCD group, A31, B8, B13, B17, Cw2, Cw6, Cw7, Drw10 and DRw12 antigens were found statistically significantly increased and Bw4 Bw6, Cw4, DR5 antigens were found statistically significantly decreased when compared with the healthy group (Table I-IV). MCD patients with frequent relapses had higher frequencies of both Cw6 (40% vs. 5% in infrequent relapsers $p < 0.005$) and DR1 (40% vs. 5% in infrequent relapsers, $p < 0.005$). Cw7 occurred in 38 percent of patients who were infrequent relapsers ($p < 0.05$) (Table V). The other antigens in these group were not different.

Table I. HLA-A in Patient and Control Groups

Antigens	MCD Group (n: 47) %	Non-MCD Group (n: 31) %	Control Group (n: 133) %			
A1	11	13	18			
A2	45	28	48			
A3	28	10	21			
A11	6	3	8			
A23(9)	0	0	1			
A24(9)	2	3	12	*$p < 0.05$	RR:1.31	CI:1.12-1.52
A26(10)	0	0	1			
A28	6	3	7			
A29	2	4	4			
A30	4	13	5			
A31	4	7	0	**$p < 0.05$	RR:5.92	CI:4.20-8.35
A32	6	0	1			
AW33	0	0	2			
Aw34	0	0	1			

HLA : human leukocyte antigen.
MCD: minimal change disease.
RR : relative risk.
CI : confidence interval.

* Demonstrates statistics with MCD patients and control.
** Demonstrates statistics with non-MCD patients and control.

Table II. HLA-B in Patient and Control Groups

Antigens	MCD Group (n:47) %	Non-MCD Group (n:31) %	Control Group (n:133) %			
B5	13	19	17			
B7	4	9	8			
B8	11	19	5	**p < 0.05	RR:3.10	CI:1.48-6.00
B12	9	6	3			
B13	26	24	5	*p < 0.0001	RR:3.08	CI:1.98-4.78
				**p < 0.001	RR:3.64	CI:1.93-6.87
B16	0	0	2			
B17	0	17	4	**p < 0.05	RR:3.16	CI:1.54-6.50
				***p < 0.005	RR:2.95	CI:2.13-4.09
B18	11	4	15			
B21	2	0	4			
B27	6	13	5			
B32	0	0	6			
B35	13	7	21			
B38(16)	0	0	6			
B39(16)	4	0	0			
B40	0	0	5			
B44	19	10	8			
B49(21)	2	3	6			
BW50(21)	2	3	0			
BW53	4	3	0			
BW55	0	3	4			
BW4	23	26	53	*p < 0.001	RR:1.35	CI:1.14-1.61
				**p < 0.05	RR:1.52	CI:1.14-2.03
BW6	34	19	60	*p < 0.05	RR:1.33	CI:1.10-1.60
				**P < 0.0005	RR:2.02	CI:1.52-2.68

HLA: human leukocyte antigen; MCD: minimal change disease; RR: relative risk; CI: confidence interval.

* Demonstrates statistics with MCD patients and control.
** Demonstrates statistics with non-MCD patients and control.
*** Demonstrates statistics with MCD patients and non-MCD patients.

Table III. HLA-C in Patient and Control Groups

Antigens	MCD Group (n:47) %	Non-MCD Group (n:31) %	Control Group (n:133) %			
Cw1	15	9	7			
Cw2	8	14	4	**p < 0.05	RR:2.72	CI:1.20-6.13
Cw3	8	16	8			
Cw4	21	10	33	**p < 0.05	RR:1.20	CI:1.06-1.36
Cw5	12	3	2	*p < 0.05	RR:2.78	CI:1.63-4.74
Cw6	12	19	5	**p < 0.05	RR:2.98	CI:1.48-6.00
Cw7	29	41	10	*p < 0.005	RR:2.40	CI:1.49-3.85
				**p < 0.0001		CI:2.33-7.74
					RR:4.25	

HLA: human leukocyte antigen; MCD: minimal change disease; RR: relative risk; CI: confidence interval.

* Demonstrates statistics with MCD patients and control.
** Demonstrates statistics with non-MCD patients and control.

Table IV. HLA-DR in Patient and Control Groups

Antigens	MCD Group (n:47) %	Non-MCD Group (n:31) %	Controls (n:133) %			
DR1	12	12	12			
DR2	6	9	22	*p < 0.05	RR:1.29	CI:1.11-1.50
DR3	8	12	11			
DR4	21	6	8	*p < 0.05	RR:2.04	CI:1.20-3.47
DR5	2	0	24	*p < 0.005	RR:1.41	CI:1.25-1.60
				**p < 0.005	RR:1.29	CI:1.17-1.41
DRw6	0	0	3			
DR7	34	19	10	*p < 0.0001	RR:3.48	CI:1.81-66.8
DRw8	2	9	3			
DRw9	3	6	6			
DRw10	4	10	1	*p < 0.05	RR:6.87	CI:1.20-39.33
				**p < 0.05	RR:3.62	CI:1.63-8.04
DRw12	0	7	0	**p < 0.05	RR:5.90	CI:4.20-8.35
DRw15(2)	10	6	1	*p < 0.05	RR:14.14	CI:1.69-118.00
DR52	31	31	23			
DR53	10	16	10			
DQ2	24	22	10	*p < 0.05	RR:1.98	CI:1.17-3.34
DQ3	21	25	21			

HLA: human leukocyte antigen; MCD: minimal change disease; RR: relative risk; CI: confidence interval.

* Demonstrates statistics with MCD patients and control.

** Demonstrates statistics with non-MCD patients and control.

Table V. HLA Frequencies in Frequent and Infrequent Relapser Groups

Antigens	Frequent Relapser Group in MCD (n:10) (%)	Infrequent Relapser Group in MCD (n:37) (%)			
Cw6	40	5	p < 0.005	RR:4.55	CI:1.79-11.55
Cw7	0	38	p < 0.05	RR:1.43	CI:1.14-1.79
DR1	40	5	p < 0.005	RR:4.55	CI:1.79-11.5

HLA: human leukocyte antigen; MCD: minimal change disease; RR: relative risk; CI: confidence interval.

Discussion

Non-MCD nephrotic syndrome may be clinically indistinguishable from MCD at onset, and renal biopsy helps in the differential diagnosis. The immunologic basis of these disorders has been the subject of a large number of studies. High prevalence of HLA DR7 has been well documented in MCD of childhood. In Australian, French, Spanish, Chinese and Arab studies with steroid responsive nephrotic syndrome (SRNS), a high prevalence of HLA DR7 was demonstrated when compared with healthy controls[4-8]. In a Turkish study from Central Anatolia, HLA DR7 and DRw53 were found more frequently in the MCD group than in the control group[9]. In our study, HLA B13, Cw5, Cw7, DR4, DR7, Drw10, DRw15(2) and DQ2 were found significantly higher than in the healthy control group. Especially HLA DR7 positivity was more predominant ($p < 0.0001$). Unlike the previous studies, not only HLA DR7, but also the distribution of other HLA groups in our nephrotic patients (MCD and non-MCD) were significantly different from the healthy controls. Although most of the studies from different regions revealed a predominance of HLA DR7 in nephrotic syndrome, varied results were reported by other study groups. In an Arab study, HLA DR2 frequency was found lower in SRNS when compared with the control group, but it was not statistically significant[8]. In our study we also observed significantly lower HLA A24(9), Bw4, Bw6, DR2, and DR5 when compared with the healthy control group.

Early studies of HLAs in nephrotic syndrome reported that HLA Bw44 was found to be

significantly more frequent in Indian nephrotic children[10]. In contrast, African children with membranous nephropathy had a significantly increased frequency of HLA Bw21[10]. Glicklich et al.[11] demonstrated that HLA DR4 was found significantly increased in FSGS patients when compared with the control group. In our study, A31, B8, B13, B17, Cw2, Cw6, Cw7, DRw10 and DRw12 antigens were found statistically significantly increased in the non-MCD dgroup when compared with the control group. HLA-B17 was found in 17 percent of the patients in the non-MCD group, but in none of the MCD patients ($p < 0.005$). Furthermore, HLA DRw12 was found in 23 percent non-MCD patients but in none of the MCD patients. Nevertheless, the difference was not statistically significant.

In a French study, Bouissou et al.[12] found significant associations with HLA DR7 and DQ2 and with the phenotypic combination HLA DR3/DR7 in the SRNS group. Significant negative associations were encountered with HLA DR2, DR6 and DQ1. The associations were stronger in frequent relapser/steroid dependent patients than in infrequent relapsers, and were not significant in non-relapsers in their study. In steroid resistant patients, the only significant association found was with the combined occurrence of HLA DR3/DR7. In our study, we found that Cw6 and DR1 were associated with frequency of relapse; in the infrequent relapsing group, Cw7 was more predominant. In a Chinese study[7], it was found that HLA DR9 was associated with the frequency of relapse and therefore with the severity of the disease. However, Ruder et al.[13] found HLA DR3 and Tümer et al.[9] found DRw53 were associated with the frequency of relapse. As Bakr[14] suggested, these differences may be explained by ethnical variations of HLA groups.

In conclusion, certain HLAs may predict the clinical course in nephrotic syndrome and may be responsible for the frequency of relapses in MCD. Further work on the ethnical variations of these factors will be worthwhile additions to the literature.

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