Human leucocyte antigens: their association with end-stage renal disease in Saudi patients awaiting transplantation

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ABSTRACT

Most patients with chronic renal failure develop end-stage renal disease (ESRD) that requires renal transplantation. This study investigates the possible associations between human leucocyte antigen (HLA) Class I and Class II molecules with ESRD. Genotyping data (HLA) obtained between 2005 and 2009 on 235 unrelated Saudi patients (147 males, 88 females; mean age: 58±7 years) with ESRD awaiting renal transplantation were assessed retrospectively at the King Khalid University Hospital. Data were compared with the results on 60 normal, healthy, unrelated Saudi individuals (37 males and 23 females; mean age: 51±5 years). HLA Class I and Class II antigens were detected by lymphocytotoxicity and a polymerase chain reaction (PCR) method using DNA sequence-specific primers. Although present in small numbers, HLA Cw2 was found in significantly fewer patients (n=11; 4.68%) compared to normal subjects (n=9; 15%) and was found to confer protection against ESRD (P=0.005; relative risk [RR]: 3.594, 95% confidence interval [CI]: 1.415–9.126). Among the HLA Class II antigens, HLA DQB1*03(8) was detected more frequently in the patient group (n=65; 27.6%) than in the normal controls (n=9; 15%) and was positively associated with risk of ESRD (P=0.04; RR: 0.462, 95% CI: 0.215–0.991). No significant differences were observed between the two groups in respect of HLA-A2, HLA-B50(21), HLA-B51(5) and HLA-Cw7 (HLA Class I), and HLA-DRB1*04, HLA-DRB1*07 and HLA-DQB1*02 (HLA Class II). Occurrence of the most frequent HLA alleles was no different between the ESRD group and the controls. The protective role of HLA-Cw2 and the marginal susceptibility associated with HLA-DQB1*03(8) for ESRD requires further investigation.

KEY WORDS: Genotyping techniques.
HLA antigens.
Kidney failure, chronic.

Introduction

Various kidney disorders leading to end-stage renal disease (ESRD) are associated more frequently with certain human leucocyte antigen (HLA) genes. For instance, idiopathic membranous glomerulonephritis is associated with HLA-DR2 in Japanese patients and with HLA-DR3 in Caucasians.[1] Gene variants of HLA-DQA1 based on single nucleotide polymorphisms have been shown to exhibit a strong association with idiopathic membranous nephropathy to an extent that these genes are now considered to predispose to the condition in those with a Caucasian ancestry.[2] Similarly, compared to the Greek population, a higher frequency of HLA DRB1*0301 has been reported in British patients with idiopathic membranous nephropathy.[3] Anti-glomerular basement membrane nephritis in Japanese patients also has a strong association with DRB1*1501.[4]

Although the presence of several HLA molecules favour predisposition to certain renal disorders, it is claimed that DRB1*07 confers resistance to renal disease.[5] HLA-DR3 and HLA-DR5 are positively associated with ESRD caused by membranous glomerulonephritis in African Americans and Caucasians; however, HLA-DR7 is believed to be negatively associated with membranous glomerulonephritis in Caucasians.[6] While chronic glomerulonephritis and hypertensive nephrosclerosis tend to exhibit a negative association with almost all A locus antigens, HLA-B58 and HLA-DRB1*03 have been shown to correlate with amyloidosis and diabetic nephropathy, respectively.[7]

These data indicate that the presence of certain HLA molecules may be associated with increased susceptibility to renal disease, while others may confer protection. Therefore, this study investigates the prevalence of Class I and Class II HLA alleles in Saudi patients with ESRD and aims to determine the disease association.

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Materials and methods

Genotyping data for 235 Saudi patients (147 males, 88 females; mean age 58±7 years) with ESRD awaiting renal transplantation at the King Khalid University Hospital between 2005 and 2009 were assessed retrospectively. The group comprised 173 (73.6%) patients with diabetic nephropathy, 37 (15.7%) patients with glomerulonephritis and 25 (10.63%) patients with essential hypertension. For the purpose of comparison, data from 60 otherwise normal,

unrelated Saudi individuals (37 males, 23 females; mean age 51±5 years) who underwent HLA genotyping during the same period were also included in the study.

Venous blood (20 mL) was collected in two acid citrate dextrose (ACD) anticoagulant tubes, and HLA typing for Class I and Class II molecules was performed by standard lymphocytotoxicity assay and a sequence-specific primer (SSP) method, respectively.

Table 1. Distribution of MHC Class I alleles in end-stage renal disease patients and healthy normal Saudi individuals.

HLA-A			HLA-B			HLA-Cw		
Allele	Normal % (*n*)	Patients % (*n*)	Allele	Normal % (*n*)	Patients % (*n*)	Allele	Normal % (*n*)	Patients % (*n*)
1	13.3 (8)	9.3 (22)	7	11.6 (7)	15.7 (37)	1	3.3 (2)	4.2 (10)
2	53.3 (32)	45.9 (108)	8	15 (9)	13.1 (31)	2	15 (9)	4.68 (11)*
3	21.6 (13)	17 (40)	13	3.3 (2)	3.4 (8)	3	10 (6)	5.9 (14)
11	8.3 (5)	5.9 (14)	18	3.3 (2)	2.9 (7)	4	18.3 (11)	20.8 (49)
23(9)	8.3 (5)	8 (19)	27	5 (3)	1.2 (3)	5	5 (3)	3.4 (8)
24(9)	25 (15)	14.4 (34)	35	10 (6)	14 (33)	6	30 (18)	39.5 (93)
25(10)	1.6 (1)	0.4 (1)	37	–	2.9 (7)	7	46.6 (28)	42.5 (100)
26(10)	5 (3)	8.5 (20)	38 (16)	6.6 (4)	4.2 (10)	8	5 (3)	6.3 (15)
29(19)	1.6 (1)	4.2 (10)	39 (16)	1.6 (1)	5.95 (14)			
30(19)	8.3 (5)	9.7 (23)	41	11.6 (7)	6.8 (16)			
31(19)	8.3 (5)	13.6 (32)	42	5 (3)	2.9 (7)			
32(19)	10 (6)	8 (19)	44 (12)	5 (3)	0.4 (1)			
33(19)	5 (3)	9.7 (23)	45 (12)	1.6 (1)	0.4 (1)			
34(10)	1.6 (1)	0.8 (2)	46	–	0.8 (2)			
36	–	0.4 (1)	47	–	–			
66(10)	–	1.2 (3)	48	–	–			
68(28)	10 (6)	18.2 (43)	49 (21)	3.3 (2)	3.8 (9)			
69(28)	–	–	69 (28)	–	–			
74(19)	1.6 (1)	3.8 (9)	50 (21)	25 (15)	24.2 (57)			
			51 (5)	30 (18)	31 (73)			
			52 (5)	1.6 (1)	2.5 (6)			
			53	6.6 (4)	7.6 (18)			
			54 (22)	–	–			
			55 (22)	5 (3)	3.4 (8)			
			56 (22)	–	–			
			57 (17)	5 (3)	0.8 (2)			
			58 (17)	5 (3)	5.5 (13)			
			59	–	–			
			60 (40)	3.3 (2)	0.4 (1)			
			61 (40)	–	0.8 (2)			
			62 (15)	3.3 (2)	0.4 (1)			
			63 (15)	6.6 (4)	3.8 (9)			
			64 (14)	–	–			
			65 (14)	3.3 (2)	5.5 (13)			
			67	–	–			
			70	5 (3)	4.2 (10)			
			73	3.3 (2)	0.4 (1)			
			75 (15)	–	1.2 (3)			
			76 (15)	–	0.4 (1)			
			77 (15)	–	0.8 (2)			
			81	–	0.4 (1)			

Normal (*n*=60); Patients (*n*=235).

*P=0.005.

HLA Class I typing

After mixing 2 mL whole blood with 100 µL thoroughly resuspended fluorobeads T (One Lambda, USA), 2 mL developer (One Lambda) was added and the contents mixed. The tube was then placed in a magnetic separator (One Lambda) and the cells (beads) were collected after discarding the supernatant and washed with calcium- and magnesium-free phosphate-buffered saline (PBS). This step was repeated and the cells were finally resuspended in 0.5 mL RPMI containing 5% heat-inactivated fetal calf serum. Finally, a cell count was performed using a fluorescence microscope and the cell concentration was adjusted to $2x10^6$/mL.

A tissue typing tray was thawed for 15 min at room temperature and 1 µL cell suspension was added to each well. Micro-droplets were mixed and the tray was incubated at room temperature for 30 min. Then, 5 µL rabbit Class I complement was added to each well and the tray incubated at room temperature for 1 h. After incubation, 5 µL fluoro quench stain was dispensed in each well and lymphocytotoxicity was assessed under ultraviolet (UV) light using a fluorescence microscope. The percentage of dead cells in each well was recorded and interpretation of the results was performed in accordance with ASHI reading standards. The results were analysed using a software program (Fluoroscan Plus, One Lambda)

HLA Class II typing

Leucocyte-rich buffy coat was collected after centrifugation of 8 mL peripheral blood at 2000 rpm for 10 min. To a 200 µL cell sample in a micro centrifuge tube was added 20 µL protease (Qiagen, Germany) and 200 µL lysing solution. After mixing, the contents were incubated at 56°C for 10 min and 200 µL ethanol (96–100%) was added to the sample and the contents were vortex-mixed. The lysate was transferred to a QIAamp spin column and centrifuged at 6000 x*g* (8000 rpm) for 1 min. DNA was collected using 200 µL elution buffer. The concentration of DNA was measured using a spectrophotometer (GeneQuant II, Pharmacia Biotech), and assessment of DNA purity was obtained by calculating the DNA and protein ratio. The DNA was then stored in buffer AE at –20°C until use.

The DNA samples were thawed at room temperature and mixed. In the negative control well, 1 µL DNA diluent and 9 µL Micro SSP D-mix containing recombinant *Thermus aquaticus* (*Taq*) polymerase (Roche Applied Biosystems; 5 units/µL) were added. In each test well, 10 µL reactants were prepared by mixing 39 µL DNA, 350 µL Micro SSP D-mix and 2 µL *Taq* polymerase, and the tray was sealed and placed in a thermocycler. After completion of the reaction cycle the PCR reactants were transferred to 2.5% agarose gel in the Micro SSP gel system. Electrophoresis was performed and the gel was photographed on a UV transilluminator. The typing results were interpreted using the worksheet provided by the manufacturer.

Statistical analysis

Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS; version 19) computer software. The differences in antigen/allele percentages between patients and controls were analysed by cross tabulation using the χ^2 test and $P \leq 0.05$ was considered significant. The strength of disease association to a particular antigen was expressed by odds ratio interpreted as relative risk (RR). This was calculated only for the antigen percentages that were significantly different between the ESRD patients and the control group.

Table 2. Distribution of MHC Class II alleles in end-stage renal disease patients and healthy normal Saudi individuals.

HLA-DRB1			HLA-DQB1		
Allele	Normal % (*n*)	Patients % (*n*)	Allele	Normal % (*n*)	Patients % (*n*)
01	3.3 (2)	9.7 (23)	02	53.3 (32)	49.7 (117)
04	21.6 (13)	34 (80)	03(7)	23.3 (14)	17 (40)
07	35 (21)	25.5 (60)	03(8)	15 (9)	27.6 (65)*
08	8.3 (5)	3.4 (8)	03(9)	3.3 (2)	1.2 (3)
09	–	–	04	5 (3)	6 (16)
10	5 (3)	6.8 (16)	05	18 (30)	23.4 (55)
11	16.6 (10)	14 (33)	06	41.6 (25)	40.8 (96)
12	1.6 (1)	1.7 (4)			
13	25 (15)	25.1 (59)			
14	1.6 (1)	2.5 (6)			
15	18.3 (11)	19.5 (46)			
16	8.3 (5)	6 (16)			
03(17)	26.6 (16)	22.1 (52)			
03(18)	3.3 (2)	3.4 (8)			

Normal (*n*=60); Patients (*n*=235).

*P=0.04.

Results

Table 1 shows the comparison between the frequency of HLA Class I alleles in 235 Saudi patients with ESRD and the 60 normal, healthy individuals in the control group. The most common alleles in both groups were HLA-A2, HLA-A68(28), HLA-A24(9), HLA-A3, HLA-B51(5), HLA-B50(21), HLA-B7, HLA-B8, HLA-Cw7, HLA-Cw6 and HLA-Cw4. Comparative analysis of the data did not reveal any significant difference between the two groups, except in the case of HLA-Cw2. This allele was detected in nine (15%) healthy individuals and 11 (4.68%) patients with ESRD. Although present in a relatively small number of individuals, the difference was significant (P=0.005) and HLA-Cw2 was found to be negatively associated with ESRD (RR: 3.594, 95% confidence interval [CI]: 1.415–9.126).

Table 2 compares the distribution of HLA Class II alleles in Saudi patients with ESRD and normal, healthy individuals. The more frequently occurring alleles in both groups were HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*13, HLA-DRB1*03(17), HLA-DQB1*02 and HLA-DQB1*06. A statistically significant difference between the proportions of Class II alleles in the ESRD patients and the healthy controls was found only for HLA-DQB1*03(8) (P=0.04). The presence of HLA-DQB1*03(8) in a higher number of patients was found to have a positive association with ESRD (RR: 0.462, 95% CI: 0.215–0.991).

Discussion

Although there was no significant difference in the most frequently occurring HLA Class I and Class II alleles between the ESRD patients and the normal controls, HLA-Cw2 was found to confer protection and HLA-DQB1*03(8) was positively associated with ESRD. Over time, the HLA-Cw locus has gained importance regarding disease association. A study from Kuwait indicates that the presence of the HLA-Cw2 allele, along with HLA-B27, is associated with an increased risk of ankylosing spondylitis and related spondyloarthropathies.[8]

Type I non-pustular psoriasis usually presents before the age of 40 and has been linked with HLA-Cw6, while type II disease presents after the age of 40 and is associated with HLA-Cw2.[9] Similarly, a study investigating the relationship between HLA-C alleles and differentiated thyroid carcinoma has linked HLA-Cw7 and HLA-Cw2 with cervical lymph node involvement.[10]

Existing data support a positive association for the HLA-Cw2 allele with various disorders, indicating its role in predisposition to certain diseases. In contrast to these observations, in the present study, the HLA-Cw2 allele appeared to confer protection against ESRD. Data supporting HLA-Cw2 as a protective allele, particularly in renal disorders, are lacking and the observations made in the present study prompt further investigations to evaluate allele-associated protection against ESRD.

Among the HLA-DR and HLA-DQ alleles assessed in the present study, HLA-DQB1*03(8) was found to be present more frequently in ESRD patients, which was associated with a marginally greater risk of having the disease. Data on HLA-DQB1*03 association with ESRD are scarce. Polymorphisms involving the HLA-DRB1 locus are associated with IgA nephropathy, which is an important cause of ESRD. The DRB1*030101 allele in particular is a predictor of disease progression and renal damage associated with IgA nephropathy.[11]

The HLA-DQB1*03 allele is associated with predisposition and protection in other disorders. Although not a risk factor independently, HLA-DQB1*03 has been shown to be associated with increased likelihood of developing ankylosing spondylitis when present with HLA-B27.[12] Similarly, the HLA-DQB1*03 allele has also been associated with increased predisposition to hepatocellular carcinoma and autoimmune gastritis.[12,13] In addition, the HLA-DQB1*03 allele is also thought to confer protection against type I diabetes mellitus.[14,15] However, association of the HLA-DQB1*03 allele with ESRD observed in the present small study needs to be investigated further.

HLA-A2 was the most common allele detected in ESRD patients (45.9%) and the normal controls (53.3%) in the present study. A study investigating the incidence of Kaposi's sarcoma after renal transplantation reported a remarkably high (83.3%) frequency of HLA-A2 among patients compared to normal individuals (43.6%).[16] In addition, a Canadian study found a higher percentage (69%) of the HLA-A2 allele in young patients with diabetic ESRD compared to normal, healthy individuals (36%), with the majority of patients showing either the HLA-A2-DR4 or HLA-DR8 haplotype.[17]

Similarly, HLA-B51(5) was the most common allele among the HLA-B locus antigens in the present study, where frequency in the ESRD patients and controls was 31% and 30%, respectively. A higher frequency of HLA-B51(5) has been associated with Behcet's disease (76.9%) in the Saudi population compared to healthy individuals (22.2%).[18]

As no significant differences were observed in the percentages of commonly occurring HLA Class I antigens between the patients and the controls in the present study, it was difficult to establish a disease association. Furthermore, the allele and haplotype distribution of Class I and Class II loci are known to be restricted by racial and geographical differences,[19] as demonstrated by reports that the HLA-A2 allele predominates among the A locus antigens in the Sudanese and Tunisian populations.[19,20] Similarly, HLA-B51(5) is also the most common HLA B locus allele among the Sudanese.[20] It is possible, therefore, that the detection of higher frequencies of HLA-A2 and HLA-B51(5), together with other HLA A and B locus alleles in the present study, may be due to a higher prevalence in the Saudi population, and are not associated with ESRD.

Owing to the strong linkage disequilibrium that exists between HLA DRB1 and DQB1 alleles, certain DRBI and DQB1 alleles and DRB1-DQB1 haplotypes have been linked with type I diabetes in various ethnic groups.[21,22] In Arab populations the DRB1030101–DQB10201 haplotype has been associated with susceptibility to type I diabetes, whereas certain HLA-DRB1 and HLA DQB1 alleles have also been shown to confer protection, depending upon the ethnicity.[23] The differential association of DRB1 and DQB1 alleles with type I diabetes prompts further investigation of ESRD patients using high-resolution PCR to identify the nature of DRB1 and DQB1 alleles conferring susceptibility or predisposition to ESRD.

The occurrence of ESRD is the final outcome of several disease processes causing renal damage. The present study fell short of providing conclusive evidence of HLA association with ESRD, and it would therefore seem to be more relevant to investigate HLA association with the specific disorders that lead to ESRD. □

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