Original Article

Human leukocyte antigen alleles, genotypes and haplotypes frequencies in renal transplant donors and recipients from West Central India

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BACKGROUND: Human leukocyte antigen (HLA) is comprised of a highly polymorphic set of genes which determines the histocompatibility of organ transplantation. The present study was undertaken to identify HLA class I and class II allele, genotype and haplotype frequencies in renal transplant recipients and donors from West Central India.

MATERIALS AND METHODS: HLA typing was carried out using Polymerase Chain Reaction-Sequence Specific Primer in 552 live related and unrelated renal transplant recipients and donors.

RESULTS: The most frequent HLA class I and class II alleles and their frequencies in recipients were HLA-A*01 (0.1685) and A*02 (0.1649), HLA-B*35 (0.1322), and HLA-DR beta 1 (DRB 1)*15 (0.2192), whereas in donors, these were HLA-A*02 (0.1848) and A*01 (0.1667), HLA-B*35 (0.1359), and HLA-DRB1*15 (0.2409). The two-locus haplotype statistical analysis revealed HLA-A*02-B61 as the most common haplotype with the frequency of 0.0487 and 0.0510 in recipients and donors, respectively. Further, among the three locus haplotypes HLA-A*33-B*44-DRB1*07 and HLA-A*02-B*61-DRB1*15 were the most common haplotypes with frequencies 0.0362 and 0.0326, respectively in recipients and 0.0236 and 0.0323, respectively in donors. Genotype frequency revealed a high prevalence of genotype HLA-A*02/A*24 in recipients (0.058) compared to donors (0.0109) whereas low prevalence of HLA-A*01/A*02 in recipients (0.0435) than in donors (0.0797). The phylogenetic and principal component analysis of HLA allele and haplotype frequency distribution revealed genetic similarities of various ethnic groups. Further, case control analysis provides preliminary evidence of association of HLA-A genotype ($P < 0.05$) with renal failure.

CONCLUSION: This study will be helpful in suitable donor search besides providing valuable information for population genetics and HLA disease association analysis.

Key words: Allele, genotype, haplotype, human leukocyte antigens frequencies, polymorphism, renal transplant

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Introduction

Renal transplant is the most common and standard therapy in case of renal failure. The success of transplantation largely depends on the compatibility of human leukocyte antigens (HLA).[1] HLA is also known as the main histocompatibility complex, which is encoded by highly polymorphic set of genes located on the short arm of chromosome 6 (Chr 6p21.1-6p21.3),[2] and play a key role in the immune system. HLA comprises two main groups; class I antigens (HLA-A, -B, -C) and class II antigens (HLA-DR, -DQ, -DP) which forms key determinants of histocompatibility. The HLA system is the most polymorphic and shows the greatest degree of polymorphism among all expressed loci in the human genome.[3,4] The polymorphic nature, tight linkage among the loci and non-random association of alleles makes HLA an engaging candidate for population genetics study.

HLA allele frequency distribution in the population may be used to study disease associations, origin and

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genetic relationship among the population and to identify useful epitopes for molecular vaccines.[5] Clinically, HLA typing is a standard practice to identify compatible donors for solid organ and bone marrow transplantation. The association of various diseases with HLA alleles has been well-established.[6] The varying frequencies of the alleles in different populations worldwide may account to some extent, for increased resistance or susceptibility to diseases. Such information is helpful in identifying differences that may lead to a better understanding of disease mechanisms.[7,8] The interaction between antigenic epitopes and the host immune system varies with the HLA alleles involved. This information is useful in selecting appropriate epitopes for inclusion in molecular vaccines and subsequently determining the efficacy of these vaccines in a particular population.[9] The HLA locus is also useful in determining the origin, migration and relationships between populations.[10]

In view of this, the present study was conducted to establish the frequencies of HLA class I and class II alleles, genotypes and haplotypes in 552 renal transplant recipients and donors from West Central India (WCI), to characterize their frequency distribution and disease association and compare with the world population to study genetic similarities among different populations.

Materials and Methods

Sample collection

Five to ten ml of venous blood samples were collected in Ethylenediaminetetraacetic acid (EDTA) vials with the consent of 552 individuals comprising of 276 renal transplant recipients and their prospective live related and unrelated donors from the west-central India and stored at 4°C.

HLA typing

The DNA from each blood sample was isolated using the method described by John, Weitzner.[11] The purity and concentration of the DNA was estimated using NanoDrop1000 spectrophotometer (Thermo scientific). All DNA samples were stored at –20°C until tested. HLA typing for HLA A, B, DRB1, DRB3, DRB4 and DRB5 alleles was carried out using Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) (Micro SSP™ Generic HLA Class I and Class II ABDR DNA Typing Tray, One Lambda Inc., Canoga Park, CA). Interpretation of the results was carried out using the worksheets provided by the manufacturer.

Statistical analysis

Statistical analysis was carried out using MP Genomics 4 software (SAS Institute Inc. USA). All loci were tested individually for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between the loci, and the statistical significance was determined using the Chi-square test. HWE and LD analysis, alleles, haplotypes and genotypes frequency analysis, principal component analysis (PCA) and case control association analysis were performed by JMP Genomics 4 software. Case control association analysis was based on Trend tests statistics.[12] The phylogenetic trees were constructed by neighbor-joining method using phylogeny inference package PHYLIP version 3.6[13] with bootstra *P* value 100.

Results

HLA-A,-B and DRB1 allelic frequencies in renal transplant recipient and donors

Fourteen HLA-A, 32 HLA-B and 13 HLA-DRB1 alleles were detected among recipients [Table 1] whereas 15 HLA-A, 29 HLA-B and 13 HLA-DRB1 alleles were detected among donors [Table 2]. The most frequent HLA-A alleles were HLA-A*01 (0.1685), HLA-A*02 (0.1649) and HLA-A*24 (0.1576), HLA-B alleles were HLA-B*35 (0.1322), HLA-B*61 (0.1033) and HLA-B*44 (0.0942) and HLA-DRB1 alleles were DRB1*15 (0.2192), DRB1*07 (0.1413) and DRB1*03 (0.1250) among recipients whereas the most frequent HLA-A alleles were HLA-A*02 (0.1848), HLA-A*01 (0.1667) and HLA-A*11 (0.1594), HLA-B alleles were HLA-B*35 (0.1359), HLA-B*61 (0.1196) and HLA-B*51 (0.0942) and HLA-DRB1 alleles were DRB1*15 (0.2409), DRB1*07 (0.1359) and DRB1*13 (0.1070) among donors [Table 3]. The HWE analysis revealed study population exists in genetic equilibrium for the HLA-A, HLA-B and HLA-DRB1 loci in donors whereas for HLA-A and HLA-B loci in recipients [Table 4]. The LD analysis revealed linkage

Table 1: Allele frequency of recipients (*n*=276)

HLA-A	Number	Frequency	HLA-B	Number	Frequency	HLA-DRB1	Number	Frequency
A*01	93	0.1685	B*13	11	0.0199	DRB1*01	17	0.0308
A*11	76	0.1377	B*15	5	0.0091	DRB1*03	69	0.125
A*02	91	0.1649	B*18	19	0.0344	DRB1*04	41	0.0743
A*23	3	0.0091	B*27	5	0.0091	DRB1*07	78	0.1413
A*24	87	0.1576	B*35	73	0.1322	DRB1*08	6	0.0109
A*26	27	0.0489	B*37	15	0.0272	DRB1*09	3	0.0054
A*29	12	0.0217	B*38	5	0.0091	DRB1*10	42	0.0761
A*03	27	0.0489	B*39	1	0.0018	DRB1*11	46	0.0833
A*30	5	0.0091	B*40	9	0.0163	DRB1*12	19	0.0344
A*31	18	0.0326	B*41	2	0.0036	DRB1*13	55	0.0996
A*32	21	0.038	B*44	52	0.0942	DRB1*14	49	0.0925
A*33	51	0.0924	B*45	1	0.0018	DRB1*15	121	0.2192
A*68	38	0.0688	B*48	3	0.0054	DRB1*16	4	0.0072
A*74	1	0.0018	B*49	1	0.0018			
			B*50	8	0.0145			
			B*51	49	0.0888			
			B*52	48	0.087			
			B*55	13	0.0236			
			B*57	29	0.0525			
			B*58	19	0.0344			
			B*60	15	0.0272			
			B*61	57	0.1033			
			B*62	13	0.0240			
			B*63	8	0.0145			
			B*65	2	0.0038			
			B*07	27	0.0489			
			B*71	5	0.0091			
			B*75	16	0.0310			
			B*77	1	0.0018			
			B*78	1	0.0018			
			B*08	35	0.0634			
			B*95	2	0.0045			

HLA: Human leukocyte antigen, DRB1: DR beta 1

Table 2: Alleles frequency of donors (*n*=276)

HLA-A	Number	Frequency	HLA-B	Number	Frequency	HLA-DRB1	Number	Frequency
A*01	92	0.1667	B*13	13	0.0236	DRB1*01	10	0.0181
A*11	88	0.1594	B*15	6	0.0109	DRB1*03	56	0.1014
A*02	102	0.1848	B*18	15	0.0272	DRB1*04	42	0.0761
A*23	3	0.0090	B*27	6	0.0109	DRB1*07	75	0.1359
A*24	77	0.1395	B*35	75	0.1359	DRB1*08	6	0.0109
A*26	23	0.0417	B*37	14	0.0254	DRB1*09	9	0.0163
A*29	11	0.0199	B*38	7	0.0127	DRB1*10	38	0.0688
A*03	36	0.0652	B*39	2	0.0036	DRB1*11	42	0.0761
A*30	5	0.0091	B*40	4	0.0072	DRB1*12	20	0.0362
A*31	16	0.029	B*41	4	0.0072	DRB1*13	58	0.1070
A*32	19	0.0344	B*44	47	0.0851	DRB1*14	57	0.1051
A*33	40	0.0725	B*45	1	0.0018	DRB1*15	133	0.2409
A*68	35	0.0634	B*48	2	0.0036	DRB1*16	4	0.0072
A*69	2	0.0036	B*49	2	0.0036			
A*74	1	0.0018	B*50	9	0.0163			
			B*51	52	0.0942			
			B*52	49	0.0888			
			B*55	14	0.0254			
			B*57	30	0.0543			
			B*58	18	0.0326			
			B*60	17	0.0308			
			B*61	66	0.1196			
			B*62	9	0.0163			
			B*63	12	0.0239			
			B*07	30	0.0543			
			B*71	5	0.0091			
			B*75	18	0.0340			
			B*77	1	0.0018			
			B*08	22	0.0399			

HLA: Human leukocyte antigen, DRB1: DR beta 1

Table 3: Allele frequency (>0.05) of recipients (*n*=276) and donors (*n*=276)

Group	HLA-A	Number	Frequency	HLA-B	Number	Frequency	HLA-DRB1	Number	Frequency
Recipients	A*01	93	0.1685	B*35	73	0.1322	DRB1*15	121	0.2192
	A*02	91	0.1649	B*61	57	0.1033	DRB1*07	78	0.1413
	A*24	87	0.1576	B*44	52	0.0942	DRB1*03	69	0.1250
	A*11	76	0.1377	B*51	49	0.0888	DRB1*13	55	0.0996
	A*33	51	0.0924	B*52	48	0.0870	DRB1*14	49	0.0925
	A*68	38	0.0688	B*08	35	0.0634	DRB1*11	46	0.0833
				B*57	29	0.0525	DRB1*10	42	0.0761
							DRB1*04	41	0.0743
Donors	A*01	92	0.1667	B*35	75	0.1359	DRB1*15	133	0.2409
	A*02	102	0.1848	B*61	66	0.1196	DRB1*07	75	0.1359
	A*11	88	0.1594	B*51	52	0.0942	DRB1*13	58	0.1070
	A*24	77	0.1395	B*52	49	0.0888	DRB1*14	57	0.1051
	A*33	40	0.0725	B*44	47	0.0851	DRB1*03	56	0.1014
	A*03	36	0.0652	B*57	30	0.0543	DRB1*04	42	0.0761
	A*68	35	0.0634	B*07	30	0.0543	DRB1*11	42	0.0761

HLA: Human leukocyte antigen, DRB1: DR beta 1

Table 4: HWE and LD analysis of HLA frequencies in recipients and donors

Genotype	HLA-A	HLA-B	HLA-DRB1	HLA-DRB3
Recipients				
HLA-A	103.26 (0.18)			
HLA-B	994.08 (0.00)	525.25 (0.18)		
HLA-DRB1	180.71 (0.09)	703.29 (0.00)	155.00 (0.00)	
HLA-DRB3	4.87 (0.98)	6.02 (1.00)	10.02 (0.61)	174.0 (0.00)
Donors				
HLA-A	114.52 (0.25)			
HLA-B	787.85 (0.00)	288.20 (1.00)		
HLA-DRB1	155.24 (0.75)	703.29 (0.00)	86.20 (0.25)	
HLA-DRB3	6.39 (0.96)	6.02 (1.00)	10.02 (0.61)	181 (0.00)

Values in the diagonal shows Chi-square (*P* value) for HWE analysis whereas values in the remaining cells indicates Chi-square (*P* value) for LD analysis, HWE: Hardy-Weinberg equilibrium, LD: Linkage disequilibrium, HLA: Human leukocyte antigen, DRB1: DR beta 1

between HLA-A and HLA-B, and HLA-B and HLA-DRB1 genotypes in donors as well as in recipients [Table 4].

Most common HLA haplotype frequency in renal transplant recipient and donors

The two locus HLA-A-B haplotype analysis revealed A*02-B*61 and A*01-B*57 haplotypes as the most common haplotypes at a frequency of 0.0487 and 0.0431, respectively among recipients; and 0.0510 and 0.0358, respectively among donors [Table 5]. The three locus HLA-A-B-DRB1 haplotype analysis revealed A*33-B*44-DRB1*07 and A*02-B*61-DRB1*15 as the most common haplotypes at a frequency of 0.0362 and 0.0326, respectively among recipients; and 0.0323 and 0.0236, respectively among donors [Table 6]. At HLA DRB3, only two individuals were having DRB3*02 alleles, whereas at HLA-DRB4 and-DRB5 only one allele was recorded. The detail summary of the two and three locus haplotype frequencies in the recipient and donor population of the present study are given in Tables 7-10.

Table 5: Two locus HLA-A and B haplotype frequency (>0.01) of renal transplant recipients and donors

Recipients			Donors		
HLA-A	HLA-B	Frequency	HLA-A	HLA-B	Frequency
A*02	B*61	0.0487	A*02	B*61	0.0510
A*01	B*57	0.0431	A*01	B*57	0.0358
A*33	B*44	0.0426	A*11	B*51	0.0320
A*02	B*35	0.0383	A*33	B*44	0.0275
A*24	B*35	0.0316	A*11	B*35	0.0263
A*33	B*58	0.0254	A*11	B*61	0.0248
A*11	B*52	0.0242	A*02	B*35	0.0245
A*24	B*52	0.0238	A*11	B*52	0.0245
A*11	B*51	0.0238	A*24	B*52	0.0218
A*26	B*08	0.0231	A*02	B*52	0.0197
A*01	B*37	0.0218	A*24	B*35	0.0196
A*11	B*35	0.0189	A*01	B*61	0.0182
A*68	B*51	0.0175	A*01	B*35	0.0172
A*68	B*35	0.0169	A*33	B*58	0.0163
A*24	B*75	0.0162	A*24	B*51	0.0160
A*11	B*44	0.0159	A*01	B*63	0.0159
A*11	B*61	0.0151	A*03	B*35	0.0157
A*01	B*61	0.0135	A*24	B*75	0.0155
A*29	B*07	0.0128	A*31	B*51	0.0134
A*24	B*44	0.0120	A*11	B*13	0.0131
A*01	B*07	0.0114	A*02	B*44	0.0130
A*31	B*51	0.0114	A*01	B*37	0.0129
A*01	B*52	0.0111	A*29	B*07	0.0127
A*24	B*60	0.0110	A*01	B*52	0.0122
A*01	B*63	0.0109	A*26	B*51	0.0122
A*24	B*62	0.0107	A*24	B*07	0.0116
A*24	B*07	0.0103	A*26	B*08	0.0115
A*11	B*18	0.0102	A*02	B*18	0.0109
A*03	B*52	0.0102	A*24	B*44	0.0105
			A*32	B*44	0.0102
			A*68	B*35	0.0102

HLA: Human leukocyte antigen

Most common HLA genotypes in renal transplant recipient and donors

There were 34 HLA-A genotypes, 32 HLA-B genotypes and 34 HLA-DRB1 genotypes in renal transplant recipients, whereas 32 HLA-A genotypes, 33 HLA-B genotypes and 36 HLA-DRB1 genotypes in renal transplant donors [Figures 1-3]. The genotypes revealing

Table 6: Three locus HLA-A, B and DRB1 haplotype frequency (>0.01) of renal transplant recipients of western central India

Recipients				Donors			
HLA-A	HLA-B	HLA-DRB1	Frequency	HLA-A	HLA-B	HLA-DRB1	Frequency
A*33	B*44	DRB1*07	0.0362	A*02	B*61	DRB1*15	0.0323
A*02	B*61	DRB1*15	0.0326	A*33	B*44	DRB1*07	0.0236
A*01	B*57	DRB1*07	0.0234	A*01	B*57	DRB1*07	0.0196
A*26	B*08	DRB1*03	0.0215	A*11	B*51	DRB1*15	0.0192
A*02	B*35	DRB1*14	0.0191	A*24	B*35	DRB1*15	0.0143
A*01	B*57	DRB1*15	0.0164	A*11	B*35	DRB1*11	0.0127
A*24	B*35	DRB1*13	0.0127	A*11	B*51	DRB1*04	0.0124
A*01	B*37	DRB1*10	0.0125	A*01	B*61	DRB1*15	0.0123
A*01	B*52	DRB1*15	0.0113	A*02	B*35	DRB1*14	0.0114
A*11	B*08	DRB1*03	0.0112	A*24	B*75	DRB1*12	0.0109
A*02	B*44	DRB1*07	0.0111	A*24	B*08	DRB1*03	0.0109
A*24	B*75	DRB1*12	0.0109	A*24	B*52	DRB1*15	0.0107
A*01	B*61	DRB1*15	0.0105	A*01	B*37	DRB1*10	0.0106
A*11	B*51	DRB1*04	0.0105	A*11	B*52	DRB1*15	0.0106
A*33	B*58	DRB1*03	0.0104				
A*68	B*51	DRB1*13	0.0102				

HLA: Human leukocyte antigen, DRB1: DR beta 1

Table 7: HLA-A and B haplotype frequencies of renal transplant recipients

HLA-A	HLA-B	Frequency	HLA-A	HLA-B	Frequency
A*02	B*61	0.0487	A*02	B*52	0.0099
A*01	B*57	0.0431	A*01	B*51	0.0098
A*33	B*44	0.0426	A*01	B*35	0.0093
A*02	B*35	0.0383	A*02	B*50	0.0091
A*24	B*35	0.0316	A*26	B*61	0.0089
A*33	B*58	0.0254	A*01	B*44	0.0083
A*11	B*52	0.0242	A*11	B*08	0.0080
A*24	B*52	0.0238	A*11	B*62	0.0079
A*11	B*51	0.0238	A*32	B*44	0.0079
A*26	B*08	0.0231	A*31	B*08	0.0078
A*01	B*37	0.0218	A*24	B*61	0.0076
A*11	B*35	0.0189	A*24	B*13	0.0075
A*68	B*51	0.0175	A*02	B*18	0.0073
A*68	B*35	0.0169	A*01	B*08	0.0073
A*24	B*75	0.0162	A*02	B*75	0.0072
A*11	B*44	0.0159	A*68	B*08	0.0068
A*11	B*61	0.0151	A*33	B*35	0.0067
A*01	B*61	0.0135	A*26	B*51	0.0063
A*29	B*07	0.0128	A*31	B*35	0.0063
A*24	B*44	0.0120	A*02	B*57	0.0057
A*01	B*07	0.0114	A*02	B*58	0.0055
A*31	B*51	0.0114	A*02	B*48	0.0055
A*01	B*52	0.0111	A*03	B*50	0.0055
A*24	B*60	0.0110	A*32	B*40	0.0055
A*01	B*63	0.0109	A*32	B*55	0.0055
A*24	B*62	0.0107	A*68	B*52	0.0052
A*24	B*07	0.0103	A*24	B*18	0.0051
A*11	B*18	0.0102	A*01	B*18	0.0050
A*03	B*52	0.0102	A*02	B*44	0.0050
			A*11	B*60	0.0050

HLA: Human leukocyte antigen

highest frequencies for HLA-A were A*11/*02, A*01/*24 and A*02/*24 [Figure 4a] and A*01/*02, A*11/*02 and A*01/*24 [Figure 4b] in recipients and donors, respectively. The genotypes with the highest frequency for HLA-B locus were B*44/*61, B*35/*52 and B*35/*61 [Figure 2a] and B*35/*51, B*35/*61, and B*44/*52 [Figure 2b] in recipients and donors, respectively; whereas for HLA-DRB1 locus were DRB1*15/*15, DRB1*07/*15 and DRB1*03/*10 [Figure 3a] and DRB1*13/*15, DRB1*14/*15 and DRB1*03/*15 [Figure 3b] in recipients and donors, respectively.

Comparison of HLA allele and haplotype frequency distribution with world population

HLA allelic and haplotype frequencies of the present study were compared with other Indian populations and worldwide populations. The allelic frequencies of other ethnic groups were obtained from http://www.allelefrequencies.net.[14] The allele frequencies that tallied to 1 and typed by PCR-SSP or sequence based typing were only considered for comparison from different geographical regions. It was observed that the HLA-A*01 allelic frequency was higher in our recipients compared to other Indian populations,[14] whereas HLA-A*02 frequency was higher in our donors but lower than other world populations. When highest frequencies of recipients and donors compared with world population, HLA-A*01 frequency was highest in our population while that of HLA-A*02 was lowest in our population [Table 11]. For HLA-B locus, the HLA-B*35 allele frequency was highest in our population for both recipients and donors, than other Indian population. Allele HLA-B*60, B*61, B*62, B*63, B*71, B*75 and B*77 were found only in our recipient and donor samples while HLA-B*65, B*78 and B*95 were found only in recipients. When highest frequencies of recipients and donors were compared with world population, HLA-B*35 frequency was highest

Table 8: HLA-A, B and DRB1 haplotype frequencies of renal transplant recipients of western central India

HLA-A	HLA-B	HLA-DRB1	Frequency	HLA-A	HLA-B	HLA-DRB1	Frequency
A*33	B*44	DRB1*07	0.0362	A*11	B*18	DRB1*15	0.0073
A*02	B*61	DRB1*15	0.0326	A*02	B*61	DRB1*10	0.0073
A*01	B*57	DRB1*07	0.0234	A*24	B*52	DRB1*14	0.0073
A*26	B*08	DRB1*03	0.0215	A*29	B*07	DRB1*10	0.0073
A*02	B*35	DRB1*14	0.0191	A*33	B*58	DRB1*13	0.0073
A*01	B*57	DRB1*15	0.0164	A*32	B*44	DRB1*07	0.0073
A*24	B*35	DRB1*13	0.0127	A*11	B*52	DRB1*15	0.0068
A*01	B*37	DRB1*10	0.0125	A*02	B*35	DRB1*03	0.0060
A*01	B*52	DRB1*15	0.0113	A*33	B*58	DRB1*15	0.0060
A*11	B*08	DRB1*03	0.0112	A*24	B*51	DRB1*13	0.0055
A*02	B*44	DRB1*07	0.0111	A*68	B*35	DRB1*11	0.0055
A*24	B*75	DRB1*12	0.0109	A*01	B*07	DRB1*08	0.0055
A*01	B*61	DRB1*15	0.0105	A*11	B*52	DRB1*14	0.0055
A*11	B*51	DRB1*04	0.0105	A*11	B*61	DRB1*14	0.0055
A*33	B*58	DRB1*03	0.0104	A*02	B*18	DRB1*15	0.0055
A*68	B*51	DRB1*13	0.0102	A*02	B*52	DRB1*11	0.0055
A*24	B*52	DRB1*15	0.0095	A*24	B*35	DRB1*15	0.0055
A*01	B*08	DRB1*03	0.0091	A*24	B*08	DRB1*03	0.0055
A*11	B*35	DRB1*15	0.0091	A*29	B*07	DRB1*03	0.0055
A*24	B*18	DRB1*11	0.0091	A*03	B*51	DRB1*15	0.0055
A*24	B*35	DRB1*10	0.0088	A*32	B*52	DRB1*15	0.00.55
A*02	B*35	DRB1*15	0.0084	A*24	B*62	DRB1*14	0.0054
A*11	B*52	DRB1*04	0.0080	A*02	B*50	DRB1*03	0.0054
A*01	B*35	DRB1*11	0.0073	A*11	B*61	DRB1*11	0.0051
A*01	B*44	DRB1*07	0.0073	A*31	B*51	DRB1*13	0.0050
A*01	B*63	DRB1*13	0.0073	A*68	B*08	DRB1*03	0.0050

HLA: Human leukocyte antigen, DRB1: DR beta 1

Table 9: HLA-A and B haplotype frequencies of renal transplant donors

HLA-A	HLA-B	Frequency	HLA-A	HLA-B	Frequency
A*02	B*61	0.0510	A*02	B*75	0.0100
A*01	B*57	0.0358	A*01	B*60	0.0098
A*11	B*51	0.0320	A*24	B*08	0.0098
A*33	B*44	0.0275	A*03	B*08	0.0094
A*11	B*35	0.0263	A*02	B*38	0.0091
A*11	B*61	0.0248	A*68	B*57	0.0089
A*02	B*35	0.0245	A*02	B*50	0.0089
A*11	B*52	0.0245	A*68	B*51	0.0086
A*24	B*52	0.0218	A*68	B*61	0.0086
A*02	B*52	0.0197	A*33	B*35	0.0082
A*24	B*35	0.0196	A*31	B*35	0.0081
A*01	B*61	0.0182	A*01	B*07	0.0080
A*01	B*35	0.0172	A*24	B*58	0.0080
A*33	B*58	0.0163	A*01	B*44	0.0078
A*24	B*51	0.0160	A*03	B*44	0.0078
A*01	B*63	0.0159	A*24	B*18	0.0073
A*03	B*35	0.0157	A*11	B*44	0.0070
A*24	B*75	0.0155	A*26	B*61	0.0069
A*31	B*51	0.0134	A*32	B*35	0.0066
A*11	B*13	0.0131	A*02	B*07	0.0065
A*02	B*44	0.0130	A*01	B*58	0.0062
A*01	B*37	0.0129	A*03	B*37	0.0060
A*29	B*07	0.0127	A*24	B*60	0.0057
A*01	B*52	0.0122	A*11	B*60	0.0056
A*26	B*51	0.0122	A*23	B*41	0.0055
A*24	B*07	0.0116	A*33	B*55	0.0055
A*26	B*08	0.0115	A*11	B*50	0.0054
A*02	B*18	0.0109	A*01	B*15	0.0054
A*24	B*44	0.0105	A*02	B*40	0.0052
A*32	B*44	0.0102	A*02	B*51	0.0052
A*68	B*35	0.0102	A*68	B*62	0.0050

HLA: Human leukocyte antigen

in Brazil mixed population [Table 12]. The HLA-DRB1*15 frequency was highest in our population from both recipients and donors, compared to other populations worldwide [Supplementary Table 13].

Phylogenetic tree analysis

The phylogenetic tree was constructed with the PHYLIP Software package by the Neighbor joining method using the allelic frequencies at HLA-A [Figure 5a], -B [Figure 5b], -DRB1 [Figure 5c] and all loci together [Figure 5d] in the West Central Indian population and other ethnic groups. The neighboring ethnic groups used in the phylogenetic tree construction were India west coast Parsi, Brazil mixed, China, England, France, Germany, Japan, Portugal, Australia and U.S.A. and our study population. The populations were included for clustering only if the frequency at a locus reached unity. The three major clusters of populations obtained based on HLA-A allelic frequencies were (1) The populations from West Central India-donor (WCI-D), U.S.An Asian and China, (2) those from France, England, Germany and (3) from India west Parsi, Brazil and Portugal. The WCI populations showed the closest genetic structure to the U.S.A, an Asian and Chinese populations. Two main clusters of populations were obtained based on HLA-B; the populations from (1) Portugal, Brazil, India, Parsi, and (2) France, Japan, China, U.S.A., an Asian,

Table 10: HLA-A, B and DRB1 haplotype frequencies of renal transplant donors of western central India

HLA-A	HLA-B	HLA-DRB1	Frequency	HLA-A	HLA-B	HLA-DRB1	Frequency
A*02	B*61	DRB1*15	0.0323	A*02	B*51	DRB1*04	0.0075
A*33	B*44	DRB1*07	0.0236	A*02	B*35	DRB1*15	0.0074
A*01	B*57	DRB1*07	0.0196	A*01	B*60	DRB1*04	0.0073
A*11	B*51	DRB1*15	0.0192	A*32	B*35	DRB1*13	0.0073
A*24	B*35	DRB1*15	0.0143	A*33	B*58	DRB1*03	0.0073
A*11	B*35	DRB1*11	0.0127	A*24	B*58	DRB1*03	0.0072
A*11	B*51	DRB1*04	0.0124	A*01	B*35	DRB1*11	0.0072
A*01	B*61	DRB1*15	0.0123	A*02	B*50	DRB1*03	0.0071
A*02	B*35	DRB1*14	0.0114	A*68	B*57	DRB1*07	0.0070
A*24	B*75	DRB1*12	0.0109	A*03	B*35	DRB1*03	0.0070
A*24	B*08	DRB1*03	0.0109	A*26	B*51	DRB1*15	0.0064
A*24	B*52	DRB1*15	0.0107	A*02	B*75	DRB1*15	0.0058
A*01	B*37	DRB1*10	0.0106	A*02	B*37	DRB1*10	0.0058
A*11	B*52	DRB1*15	0.0106	A*11	B*44	DRB1*07	0.0057
A*24	B*07	DRB1*15	0.0092	A*68	B*51	DRB1*15	0.0056
A*01	B*63	DRB1*13	0.0091	A*02	B*61	DRB1*14	0.0056
A*02	B*18	DRB1*15	0.0091	A*11	B*13	DRB1*07	0.0055
A*02	B*44	DRB1*07	0.0091	A*11	B*08	DRB1*03	0.0055
A*26	B*08	DRB1*03	0.0091	A*02	B*52	DRB1*15	0.0055
A*02	B*61	DRB1*10	0.0091	A*23	B*41	DRB1*07	0.0055
A*32	B*44	DRB1*07	0.0088	A*24	B*18	DRB1*11	0.0055
A*11	B*61	DRB1*15	0.0087	A*24	B*51	DRB1*13	0.0055
A*24	B*52	DRB1*10	0.0086	A*31	B*51	DRB1*11	0.0055
A*11	B*61	DRB1*11	0.0083	A*33	B*58	DRB1*13	0.0055

HLA: Human leukocyte antigen, DRB1: DR beta 1

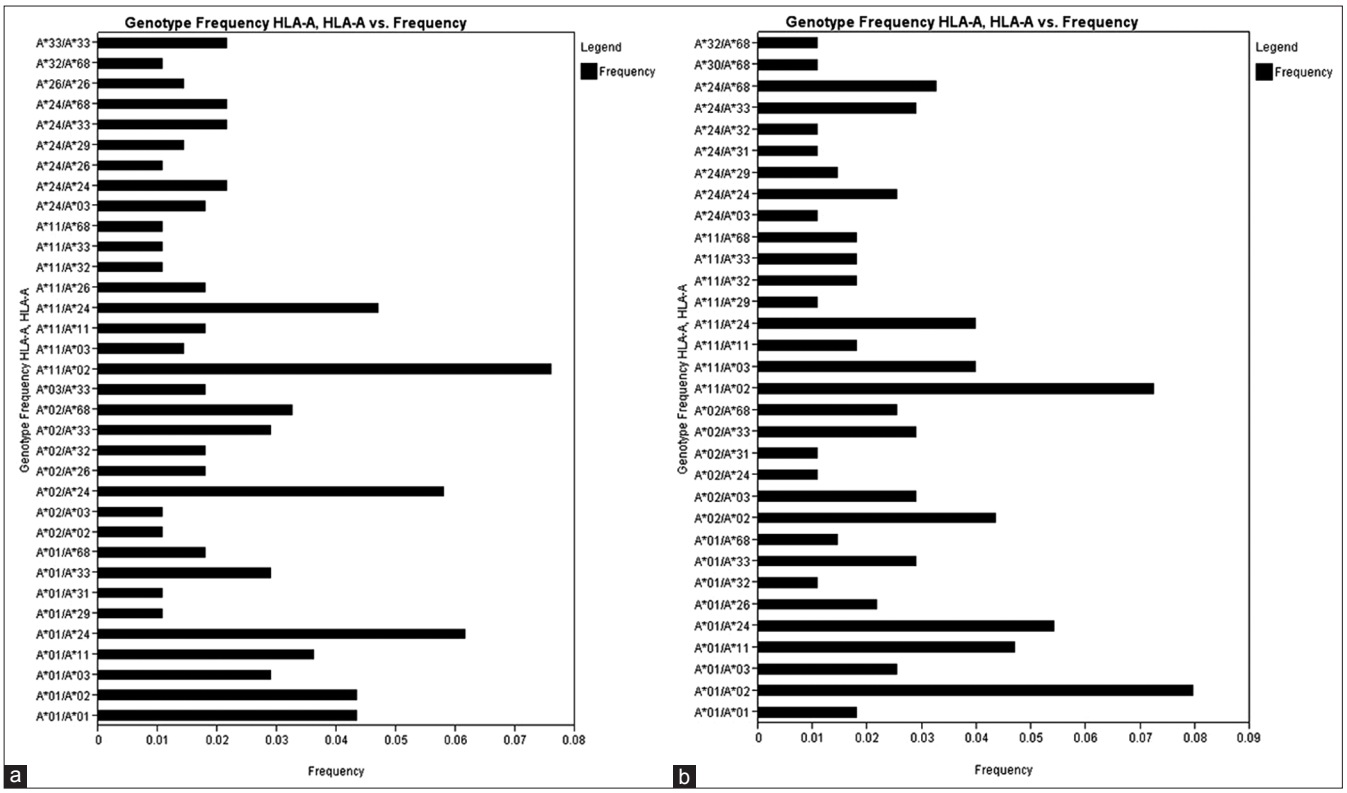

Figure 1: Human leukocyte antigen-A genotype frequencies of renal transplant recipients (a) and donors (b)

Germany and England. The WCI population showed equal genetic distance to the other populations. The two main clusters of populations obtained, based on HLA-DRB1 were the populations living in (1) U.S.A., an Asian, China, Japan, Australia, and (2) Portugal, Brazil, Germany and France. The WCI population showed the equal genetic structure to the other populations. When considered all loci (HLA-A, B and DRB1) together, the three main clusters of populations obtained were (1) from Japan, (2) from Portugal, U.S.A, an Asian, China,

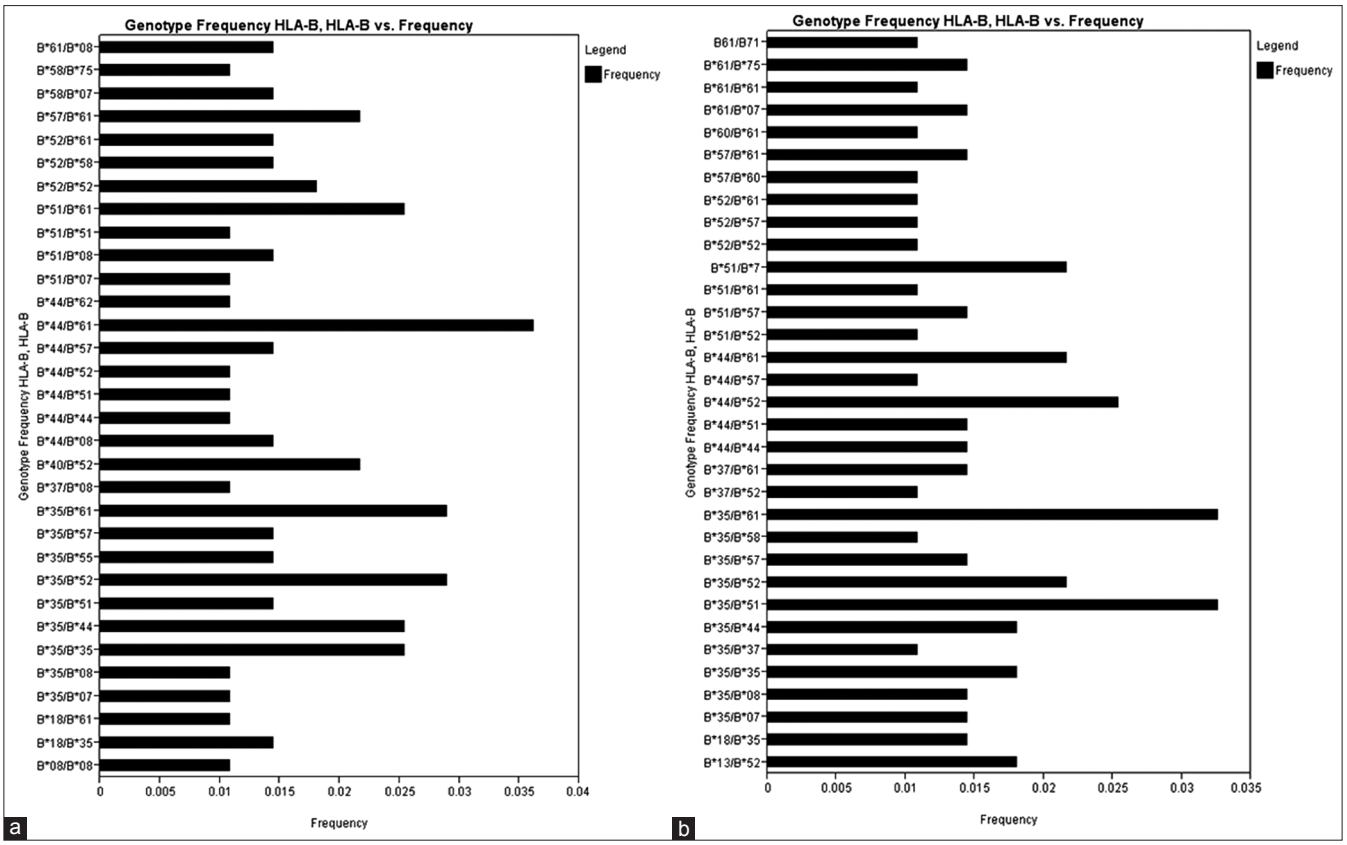

Figure 2: Human leukocyte antigen-B genotype frequencies of renal transplant recipients (a) and donors (b)

and (3) from Brazil, France and Germany. The WCI population first clustered with Japan and then other groups.

PCA

To further investigate possible genetic relationships between these populations, we performed PCA of the genotypic frequencies of HLA-A,-B and-DRB1 loci which resulted in the clustering of nine populations into three groups. The total variance of 71.5% among all population could be explained by three principal components PCA1 (31.6%), PCA2 (25.9%) and PCA3 (14.0%) [Figure 6]. Japan, China and USA Asian formed one cluster whereas our populations West Central India-recipients and WCI-D clustered with European populations (i.e., Germany, France and Portugal). Brazilian population stood apart.

Case control association analysis

Further we studied the association between HLA and disease condition. Case control association analysis between unrelated healthy donors and kidney recipients [Table 14] showed that genotype, alleles or trend in HLA A, B, and DRB1 frequencies did not show any significant difference as revealed by Chi-square test. However, Z Recessive (*P* value 0.043) and Z Max (*P* value 0.042) showed significant association of HLA-A genotypes with disease. The frequency distribution analysis of the homozygous recessive alleles in recipient revealed significantly higher frequency of HLA A*01/A*01 and A*33/A*33 genotypes in recipients (4.36% and 2.18%, respectively) compared to unrelated healthy donors (1.06% and 0.00%, respectively). Z Max indicates frequency variation in large frequency genotype at HLA locus that is associated with disease. The analysis of large frequency genotypes revealed genotypes HLA-A*11/A*02, A*01/A*24, A*02/A*24, A*11/A*24, A*01/A*01, A*01/A*02 with 7.46, 5.97, 5.97, 4.85, 4.48 and 4.48% frequencies, respectively in recipients and 12.77, 5.32, 1.06, 3.19, 1.06, and 9.57% frequencies, respectively in unrelated healthy donors.

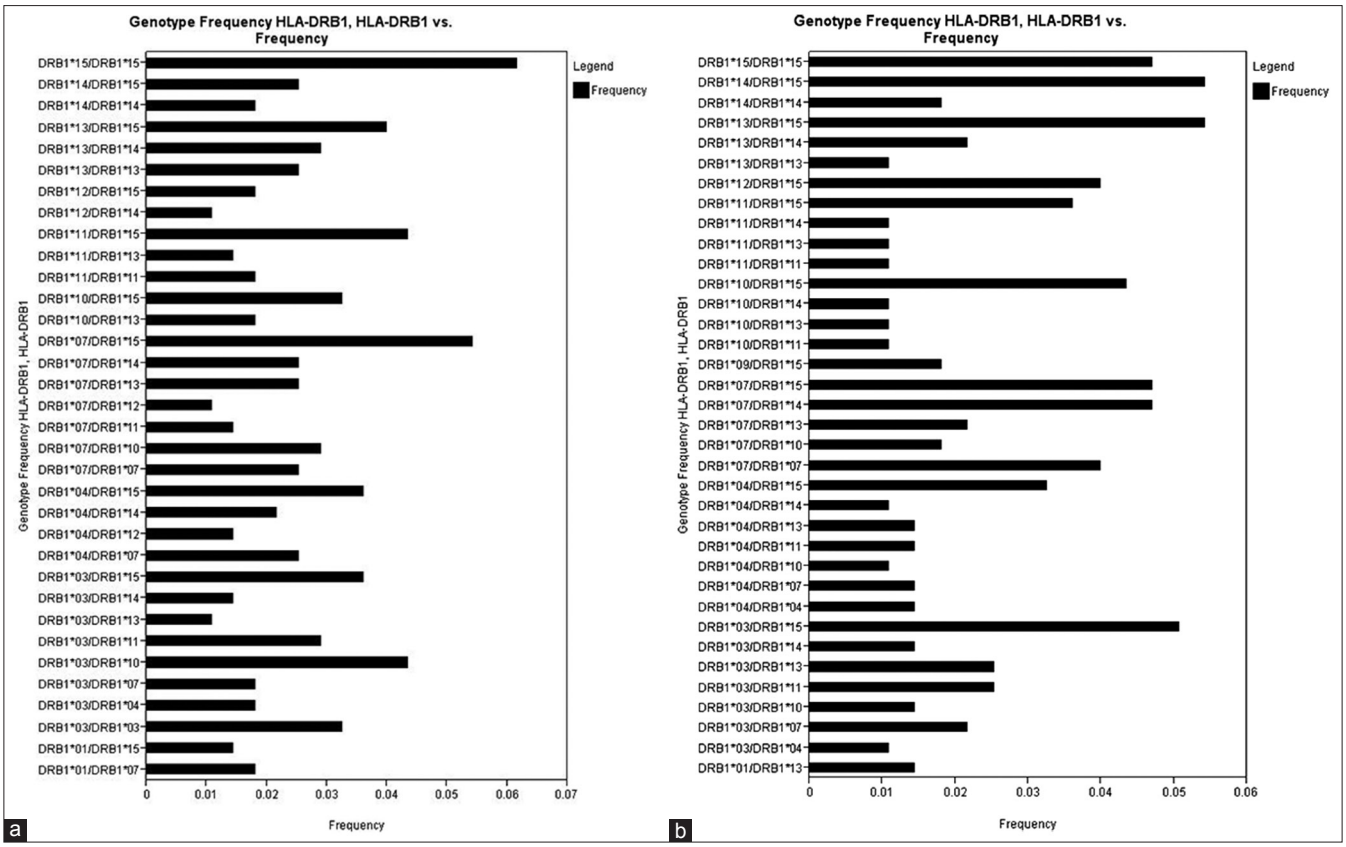

Figure 3: Human leukocyte antigen-DRB1 genotype frequencies of renal transplant recipients (a) and (b) donors

Discussion

This study describes the analysis of HLA antigen frequency distribution in kidney recipients and their prospective live related and unrelated donors from West-Central regions of India for the first time. The HLA frequency of the study population was compared with the frequency of the world population and genetic relationship between various populations were analyzed. Further, attempts were made to establish possible association of HLA alleles with kidney failure in renal transplant recipients.

In our study, the high frequency alleles in recipients and donors were HLA-A*01, *02, *24, *11; B*35, *61, *44, *51, *52; DRB1*15, *07, *03 and HLA-A*02, *01, *11, *24; B*35, *61, *51, *52, *44; DRB1*15, *07, *03, respectively. These results suggest that the frequency distribution of alleles in the recipient and donor population did not vary significantly, although relative occurrence of the alleles differed with in the groups. The two locus and three locus haplotype distribution revealed HLA-A*02-B*61, A*01-B*57, and HLA-A*33-B*44-DRB1*07, A*02-B*61-DRB1*15, as the common haplotypes in both donors as well as recipients population. However, the analysis of genotype frequency distribution in recipients and donors suggested, appreciable variation as frequency of genotype HLA-A*02/*24 was higher in recipient group (0.058) compared to donor group (0.0109). Similarly, the frequency of genotype HLA-A*01/*02 was low in recipient group (0.0435) compared to donor group (0.0797). When compared with the other Indian population, the allelic and haplotype frequency appeared to vary from the other Indian population from Maharashtra.[1] Similarly, when compared with the world population, the allelic frequency distribution varied between different ethnic groups. While we observed our study population in genetic equilibrium for HLA-A, HLA-B and HLA-DRB1 loci in donors and HLA-A and HLA-B loci in recipients, there exist genetic disequilibrium for HLA-DRB3 locus in donors and HLA-DRB1 and DRB3 loci in recipients. The linkage analysis suggested strong

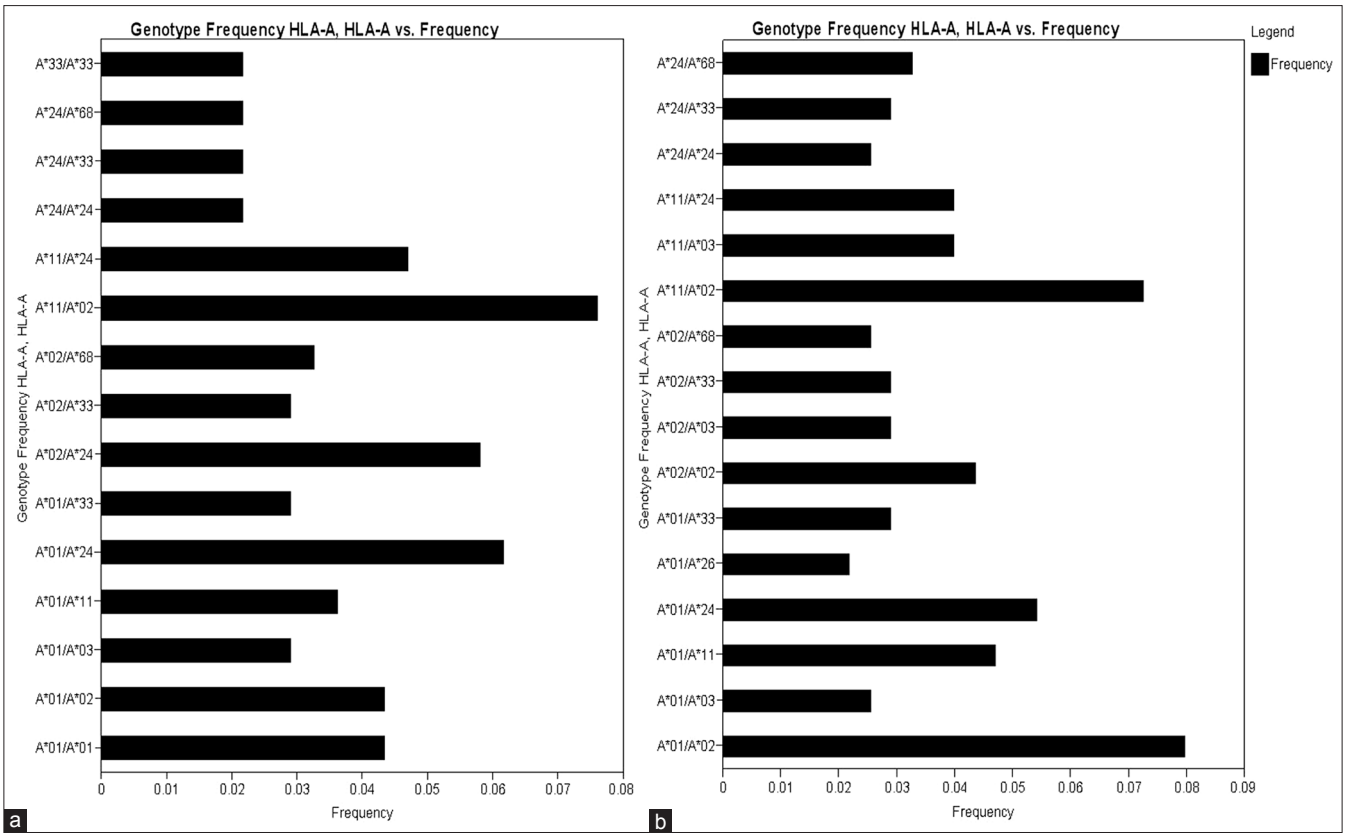

Figure 4: Human leukocyte antigen (HLA) HLA-A/A genotype frequency (>0.02) of renal transplant recipients (a) and (b) donors

Table 11: Allele frequencies of HLA-A locus in renal transplant recipients and donors of western-central India, other Indian and world population

HLA-A	WCI-R (*n*=276)	WCI-D (*n*=276)	India west coast parsi (*n*=50)	Brazil mixed (*n*=108)	China Beijing Shijiazhuang (*n*=618)	England Northwest (*n*=298)	France Southeast (*n*=130)	Germany Pop6 (*n*=8862)	Japan Pop3 (*n*=1018)	Portugal center (*n*=50)	U.S.A Asian Pop2 (*n*=2160)
A*01	0.1685	0.1667	0.09	0.091	0.036	0.21	0.159	0.154	0.002	0.13	0.053
A*02	0.1649	0.1848	0	0.33	0.339	0.309	0.24	0.299	0.243	0.28	0.2
A*03	0.0489	0.0652	0.02	0.071	0.035	0.143	0.139	0.161	0.003	0.11	0.035
A*11	0.1377	0.1594	0.04	0.091	0.247	0.07	0.049	0.051	0.104	0.06	0.204
A*23	0.0091	0.009	0	0.02	0.008	0.02	0.012	0.022	0	0.01	0.001
A*24	0.1576	0.1395	0.11	0.018	0.128	0.077	0.114	0.09	0.464	0.09	0.226
A*25	0	0	0.02	0.005	0.001	0.025	0.008	0.024	0	0	0.001
A*26	0.0489	0.0417	0.04	0.039	0.029	0.02	0.043	0.031	0.116	0.02	0.055
A*29	0.0217	0.0199	0.04	0.03	0.01	0.003	0.055	0.026	0	0.09	0.018
A*30	0.0091	0.0091	0.19	0.06	0.063	0.017	0.039	0.019	0.001	0.04	0.029
A*31	0.0326	0.029	0.18	0	0.007	0.029	0	0.023	0	0	0.035
A*32	0.038	0.0344	0.05	0.02	0.01	0.029	0.072	0.037	0	0.04	0.014
A*33	0.0924	0.0725	0.2	0.04	0.066	0.008	0.023	0.012	0.067	0.03	0.103
A*34	0	0	0	0.025	0	0	0.004	0	0	0	0.02
A*36	0	0	0	0.09	0	0	0	0	0	0	0
A*43	0	0	0	0	0	0	0	0	0	0	0
A*66	0	0	0.02	0.01	0.005	0.008	0.004	0.005	0	0.02	0.001
A*68	0.0688	0.0634	0	0.04	0.008	0.028	0.035	0.044	0	0.07	0.003
A*69	0	0.0036	0	0	0.001	0	0.004	0.002	0	0.01	0.001
A*74	0.0018	0.0018	0	0.015	0.007	0.007	0	0	0	0	0
A*80	0	0	0	0.005	0	0	0	0	0	0	0.001

WCI-R: Western centralIndia-recipients, WCI-D: Western central India-donors, HLA: Human leukocyte antigen

linkage between HLA-A and -B genotype as well as HLA-B and -DRB1 genotype but not between HLA-A and -DRB1 genotypes in both donors and recipients. This suggests there is high co-segregation of HLA-A and -B, and HLA-B and -DRB1 alleles but independent assortment of HLA-A and -DRB1 alleles.

Table 12: Allele frequencies of HLA-B locus in renal transplant recipients and donors of western-central India and other Indian and world population

HLA-B	WCI-R (*n*=276)	WCI-D (*n*=276)	India west cost parsi (*n*=50)	China Beijing Shijiazhuang Trianjian Han (*n*=618)	England Northwest (*n*=298)	France Southeast (*n*=130)	Germany (*n*=8862) Pop6	Japan Pop3 (*n*=1018)	Portugal center (*n*=50)	U.S.A Asian Pop2 (*n*=2160)	Brazil mixed (*n*=108)
B*07	0.0489	0.0543	0.0300	0.0460	0.1700	0.0840	0.1440	0.0570	0.0800	0.0470	0.0070
B*08	0.0634	0.0399	0.0100	0.0070	0.1540	0.0890	0.1040	0.0000	0.0400	0.0150	0.1350
B*13	0.0199	0.0236	0.1900	0.0960	0.0070	0.0190	0.0310	0.0150	0.0100	0.0550	0.0000
B*14	0.0000	0.0000	0.2600	0.0010	0.0100	0.0479	0.0230	0.0000	0.0400	0.0050	0.0900
B*15	0.0091	0.0109	0.0500	0.1690	0.0720	0.0790	0.0870	0.1100	0.0800	0.1330	0.0700
B*18	0.0344	0.0272	0.0000	0.0050	0.0450	0.0470	0.0480	0.0000	0.1000	0.0130	0.0670
B*27	0.0091	0.0109	0.0000	0.0190	0.0420	0.0230	0.0440	0.0030	0.0100	0.0200	0.0070
B*35	0.1322	0.1359	0.1200	0.0500	0.0440	0.0780	0.0950	0.0790	0.1400	0.0780	0.1900
B*37	0.0272	0.0254	0.0000	0.0090	0.0000	0.0310	0.0130	0.0040	0.0200	0.0150	0.0000
B*38	0.0091	0.0127	0.0000	0.0290	0.0000	0.0190	0.0190	0.0020	0.0000	0.0410	0.0090
B*39	0.0018	0.0036	0.0000	0.0320	0.0070	0.0230	0.0200	0.0370	0.0100	0.0190	0.0700
B*40	0.0163	0.0072	0.0900	0.1360	0.0650	0.0630	0.0620	0.1920	0.0300	0.1490	0.0210
B*41	0.0036	0.0072	0.0000	0.0020	0.0020	0.0150	0.0090	0.0000	0.0000	0.0010	0.0000
B*42	0.0000	0.0000	0.0000	0.0010	0.0000	0.0090	0.0000	0.0000	0.0100	0.0000	0.0290
B*44	0.0942	0.0851	0.1400	0.0380	0.2500	0.0870	0.1270	0.0650	0.1600	0.0520	0.1190
B*45	0.0018	0.0018	0.0000	0.0000	0.0050	0.0160	0.0040	0.0000	0.0500	0.0020	0.0000
B*46	0.0000	0.0000	0.0000	0.0970	0.0000	0.0000	0.0000	0.0500	0.0000	0.0590	0.0000
B*47	0.0000	0.0000	0.0000	0.0000	0.0050	0.0040	0.0030	0.0000	0.0100	0.0000	0.0000
B*48	0.0054	0.0036	0.0000	0.0240	0.0020	0.0000	0.0010	0.0270	0.0000	0.0200	0.0090
B*49	0.0018	0.0036	0.0000	0.0020	0.0070	0.0400	0.0150	0.0000	0.0100	0.0010	0.0140
B*50	0.0145	0.0163	0.0000	0.0060	0.0190	0.0161	0.0100	0.0000	0.0000	0.0070	0.0140
B*51	0.0888	0.0942	0.0300	0.0670	0.0470	0.0610	0.0580	0.0870	0.1200	0.0780	0.0070
B*52	0.0870	0.0888	0.0000	0.0170	0.0100	0.0160	0.0070	0.1090	0.0100	0.0360	0.0000
B*53	0.0000	0.0000	0.0200	0.0000	0.0000	0.0200	0.0040	0.0000	0.0100	0.0010	0.0290
B*54	0.0000	0.0000	0.0000	0.0320	0.0000	0.0000	0.0000	0.0880	0.0000	0.0310	0.0000
B*55	0.0236	0.0254	0.0100	0.0400	0.0230	0.0310	0.0180	0.0200	0.0100	0.0220	0.0000
B*56	0.0000	0.0000	0.0200	0.0050	0.0080	0.0000	0.0080	0.0120	0.0000	0.0100	0.0000
B*57	0.0525	0.0543	0.0000	0.0120	0.0000	0.0520	0.0340	0.0000	0.0300	0.0220	0.0210
B*58	0.0344	0.0326	0.0300	0.0460	0.0060	0.0300	0.0090	0.0040	0.0200	0.0580	0.0290
B*59	0.0000	0.0000	0.0000	0.0010	0.0000	0.0000	0.0000	0.0390	0.0000	0.0070	0.0000
B*60	0.0272	0.0308	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*61	0.1033	0.1196	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*62	0.0240	0.0163	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*63	0.0145	0.0239	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*65	0.0038	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*67	0.0000	0.0000	0.0000	0.0080	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0000
B*71	0.0091	0.0091	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*73	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0030	0.0000	0.0000	0.0000	0.0000
B*75	0.0310	0.0340	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*77	0.0018	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B*78	0.0018	0.0000	0.0000	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0490
B*81	0.0000	0.0000	0.0000	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000
B*82	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0140
B*95	0.0045	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

WCI-R: Western central India-recipients, WCI-D: Western central India-donors, HLA: Human leukocyte antigen

The phylogenetic trees when constructed using HLA-A, B, DRB1 frequencies independently or pooled over all loci revealed that WCI populations belonged to the Asian populations and was most closely related to other Asian groups, than to European groups. The geographically separated populations are more likely to subject to selection in distinctive environments;[15] hence genetic distance fairly correlates with the geographic distance. Genetic distance here refers to the genetic divergence between populations within a species. Small genetic distance indicates a close genetic relationship between the two populations, whereas a large genetic distance indicates a distant genetic relationship.[16] USA Asian and China always clustered together indicating that USA Asian population consists largely of Chinese (immigrants). Parsis from Indian west coast is originally migrants from the Mediterranean hence it is natural that they may not cluster with Asian populations.

The phylogenetic tree cannot properly analyze the genetic relationship among the population as several populations can be derived from a single

Table 13: Allele frequencies of HLA-DRB1 locus in renal transplant recipients and donors of western-central India and other Indian and world population

HLA-DRB1	WCI-R (*n*=276)	WCI-D (*n*=276)	Australia yuendumu aborigine (*n*=191)	Brazil mixed (*n*=108)	China	France Southeast (*n*=130)	Germany Pop6 (*n*=8862)	Japan Pop3 (*n*=1018)	Portugal center (*n*=50)	U.S.A Asian Pop2 (*n*=2160)
DRB1*01	0.0308	0.0181	0.0000	0.0900	0.0280	0.1100	0.1130	0.0790	0.0400	0.0270
DRB1*03	0.1250	0.1014	0.0000	0.1360	0.0640	0.0850	0.1090	0.0010	0.1600	0.0550
DRB1*04	0.0743	0.0761	0.0800	0.1300	0.1800	0.1600	0.1340	0.3300	0.1400	0.1430
DRB1*07	0.1413	0.1359	0.0000	0.0750	0.1000	0.0790	0.1210	0.0030	0.1400	0.0830
DRB1*08	0.0109	0.0109	0.2660	0.0700	0.0820	0.0370	0.0340	0.1440	0.0700	0.0710
DRB1*09	0.0054	0.0163	0.0000	0.0200	0.0040	0.0000	0.0100	0.0000	0.0000	0.1030
DRB1*10	0.0761	0.0688	0.0000	0.0100	0.0150	0.0090	0.0070	0.0040	0.0000	0.0310
DRB 1*11	0.0833	0.0761	0.0000	0.1300	0.0760	0.1500	0.1160	0.0290	0.1100	0.0580
DRB1*12	0.0344	0.0362	0.1200	0.0150	0.1200	0.0170	0.0190	0.0790	0.0000	0.1020
DRB1*13	0.0996	0.1051	0.0000	0.1250	0.0630	0.1650	0.1310	0.0700	0.1800	0.0670
DRB1*14	0.0925	0.1070	0.4950	0.0400	0.0740	0.0510	0.0290	0.0960	0.0200	0.0770
DRB1*15	0.2192	0.2409	0.0390	0.1240	0.1570	0.0980	0.0150	0.1600	0.1100	0.1650
DRB1*16	0.0072	0.0072	0.0000	0.0350	0.0370	0.0390	0.0260	0.0050	0.0300	0.0180

WCI-R: Western central India-recipients, WCI-D: Western central India-donors, HLA: Human leukocyte antigen, DRB1: DR beta 1

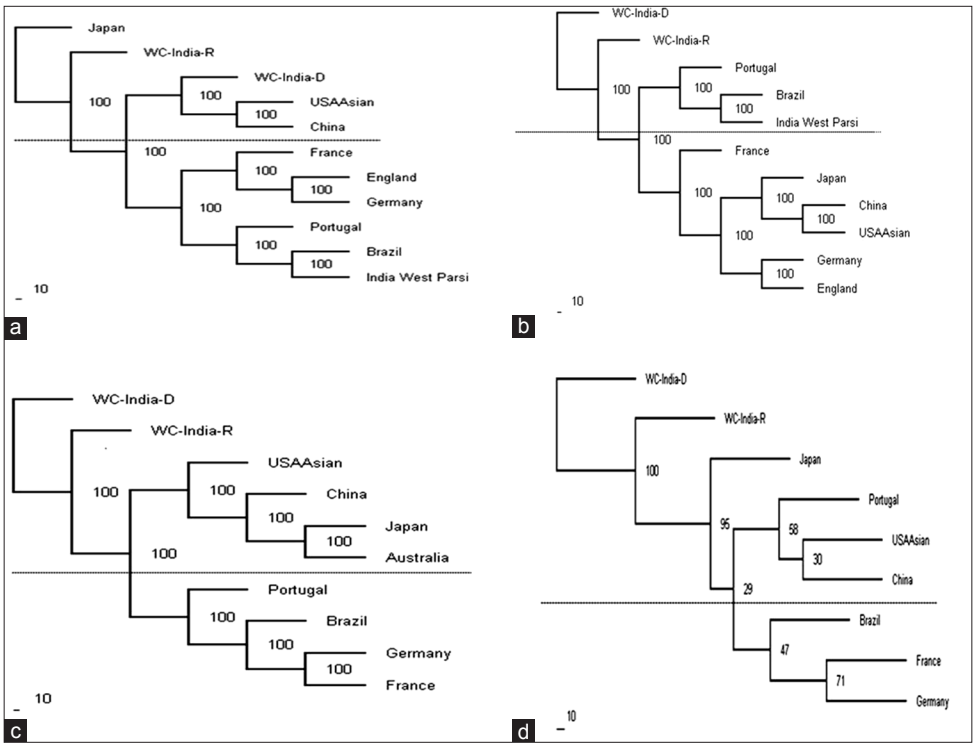

Figure 5: Dendrogram constructed by the neighbor-joining method (a) relationships of the West Central India (WCI populations with other nine populations based on the allelic frequencies of human leukocyte antigen (HLA) HLA-A locus; (b) relationships of the WCI population with other 9 populations based on the allelic frequencies of HLA-B locus; (c) relationships of the WCI population with other 8 populations based on the allelic frequencies of HLA-DRB1 locus; and (d) relationships of the WCI population with other 7 populations based on the combined allelic frequencies of HLA-A, B and DRB1 loci

population.[17] PCA can be applied to overcome these problems as an alternative to phylogenetic trees. In our study, the PCA revealed clustering of WCI population with European populations, whereas Brazilian population was completely separated. Closeness of WCI with European population revealed by PCA analysis may be better indicative of lineage as the study population and European populations are believed to be the descendent of common ancestors.

The frequency distribution analysis of HLA alleles may provide significant insights into HLA disease association.[18,19] In our study, the case control analysis between the

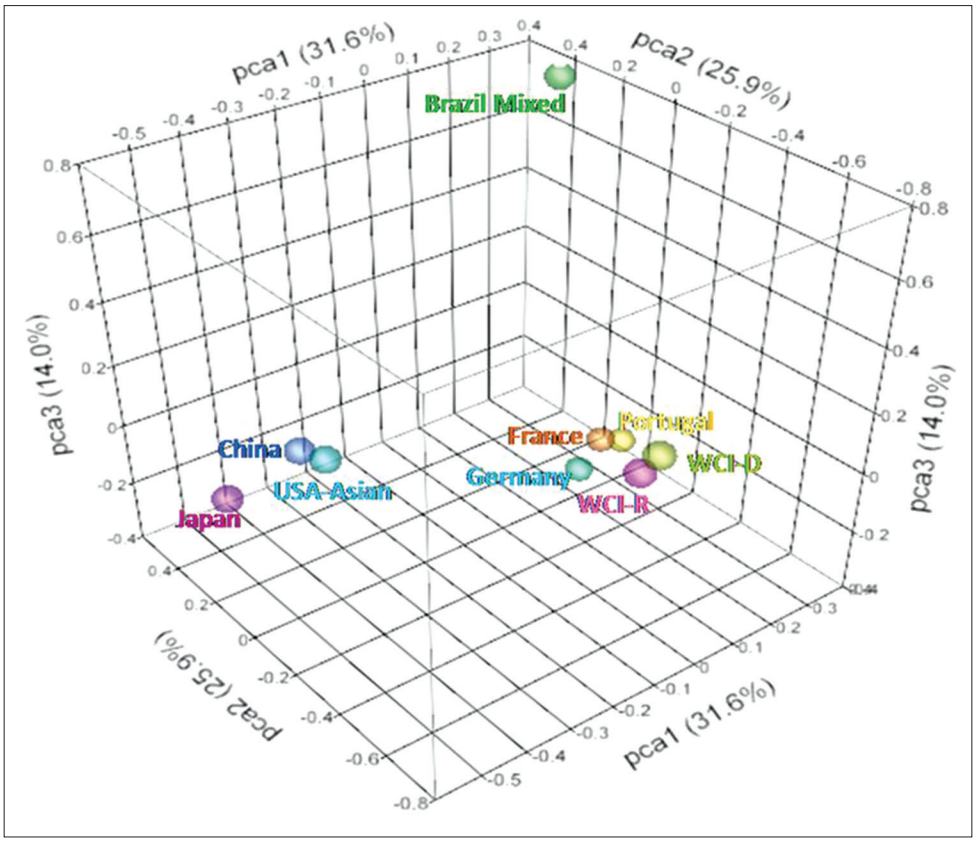

Figure 6: Principal component analysis of genotypic frequencies of HLA-A,-B and-DRB1 loci. Total variance was partitioned in to three components. Three-dimensional scattered plot showing clustering of different populations into three groups

Table 14: Case control association

Particulars	HLA-A	HLA-B	HLA-DRB1
Unrelated donors (healthy)	94	94	94
Recipients (kidney disease)	268	268	268
Genotype			
χ^2 test	64.48	141.09	58.98
Degree of freedom	67	153	64
P value	0.56	0.74	0.65
Allele			
χ^2 test	12.57	24.26	14.43
Degree of freedom	13	31	12
P value	0.48	0.79	0.27
Trend			
χ^2 test	11.64	22.64	12.81
Degree of freedom	13	31	12
P value	0.55	0.86	0.38
Dominant			
Z dom	1.899	0.259	0.357
P value	0.057	0.795	0.720
Recessive			
Z rec	2.015	0.675	1.258
P value	0.043	0.499	0.208
Max			
Z max	2.346	0.675	1.258
P value	0.0423	0.74	0.381

Z max indicates frequency variation in large frequency genotypes, HLA-A locus shows significant association with disease, HLA: Human leukocyte antigen, DRB1: DR beta 1

unrelated healthy donors and recipients suggested no significant association of HLA alleles, genotypes or trends. However, Z Recessive and Z Max analysis suggested significant association of HLA-A genotype with kidney disease. Interestingly, HLA-A*01/A*01, and -A*33/A*33 genotypic frequencies were found to be significantly higher in recipients compared to unrelated donors. The presence of these genotypes in homozygous condition may predispose such individuals to renal disease. Further, distribution of large frequency genotypes (HLA-A*11/A*02, A*02/A*24, A*01/A*01, and A*01/A*02) were also varied significantly between recipients and unrelated donors suggesting their possible association with renal disease. The association of HLA antigens with the renal diseases has been poorly established. However, it is possible that specific HLA antigens may predispose an individual to other autoimmune or infectious diseases which affect the renal function.[20-22]

Conclusion

The present study analyzed genotype, allele, and

haplotype frequency distribution in the renal transplant donors and recipients and its comparison with different populations worldwide. The information on HLA allelic distribution and genetic similarity between various populations will be useful to identify compatible donors within a specific ethnic group for patients requiring renal transplant. The HLA disease association study provides preliminary evidence of a possible association of HLA-A genotype with renal diseases. However, it also indicates the necessity for high resolution HLA genotyping to study association with the renal failure as well as to promote greater compatibility in the area of transplants. Nevertheless, the data generated in the present study represents an important source of information for investigators in the field of transplantation, HLA disease association and population genetics.

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