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HLA Antigens and Haplotype Frequencies among Renal Transplant Recipients and Donors Presenting to a Tertiary Care Hospital in Hyderabad, Telangana, South India

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Abstract

Histocompatibility testing of HLA-A, HLA-B and HLA-DRB1 antigens has great importance in the selection of kidney recipient candidates and donors for transplantation. DNA typing of HLA Loci HLA-A,B and DRB1 loci was performed using the SSO typing kit (Luminex) for 2000 renal transplant recipients and donor who presented to our tertiary care centre .There was male preponderance among recipients and female preponderance among donors.The antigen frequency of HLA A*33:01,B*44:03, B*15:18, B*57:03 were significantly higher in donors suggesting that these antigens may have protective influence on the kidney, while antigen frequency of B*57:01was significantly higher in recipients. Two locus haplotypes of HLA A-B,A-DRB1,B-DRB1 documented among recipients was A*24:02,B*51:01, A*01:01-B*52:01, A*68:01-DRB1*14:01,B*49:01-DRB1*13:02 and among donors was A*68:01-DRB1*13:01, A*03:01-B*35:01,B*44:03-DRB1*15:02,B*13:01-DRB1*07:01.Three locus HLA A- B-DRB1 haplotypes documented among recipients were A*24:02-B*40:06-DRB1*15:01 and A*01:01-B*57:01-DRB1*07:01, suggesting that these antigens may be risk factor for renal failure. Two locus and three locus haplotypes reported in our study are not reported from other parts of India. The HLA antigens and haplotypes reported in our study will help in understanding the Indian population genetics and help the organ transplant team to find suitable matched donors for transplant recipients.

Keywords: *HLA antigens , Haplotype, Luminex –SSOP, Renaltransplantation.*

Introduction

Histocompatibility testing of Human leukocyte antigen (HLA), HLA-A, HLA-B and HLA-DRB1 antigens has great importance in the selection of kidney recipient candidates and donors for transplantation [1]. HLA- A,-B and –DR mismatch plays a major role in graft loss [2].

HLA typing was previously performed by serological method by using HLA antiserum [3,4] but this procedure is now considered to be cumbersome and lacks the resolving power. With the advent of molecular typing using polymerase chain reaction (PCR) and sequence specific oligonucleotide probes (SSOPs), it has become possible to further define HLA polymorphism at the DNA level [5]. Recently, PCR and the Luminex microbead system for the simultaneous multiplex assay of amplicons hybridized to SSOP in a single detection solution have been described for high-throughput single nucleotide polymorphism (SNP) typing of HLA [6,7] Information of HLA antigens is helpful in identifying differences that may lead to a better understanding of disease mechanisms [8] and also useful in determining the origin, migration and relationships between populations[9].

Not much documented studies have been reported currently on frequency distribution of HLA antigens among renal transplant recipients and donors from Telangana region of South Indian population.With

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this background, the present study was undertaken to establish HLA class I and class II antigens and haplotype frequencies by Lifecodes ® HLA –SSO typing kits which combines the PCR amplification–SSO protocol with xMAP technology developed by Luminex Corporation as the analytical technique in 2000 live renal transplant recipients and donors , presented to tertiary care centre from Hyderabad, Telangana, South India.

Materials and Method

A total of 2000 renal transplant recipients and donors who presented to our tertiary care centre were retrospectively included in the study. For each recipient and donor ,5 ml whole blood was collected in *BD Vacutainer® EDTA* blood collection tubes. DNA was extracted from the blood samples by using the Wizard genomic *DNA purification* kit from *Promega* Madison WI as per manufacturer's protocol. DNA typing of HLA Loci HLA-A, HLA-B and HLA- DRB1 loci was performed on a Multi-Analyte Profiling system (xMAP) (Luminex HLA-SSO) using Lifecodes ® HLA –SSO typing kit (Immucor, Stamford, CT, USA) as per the manufacturers protocol.

The significance of the distribution of antigens between the recipient and donor was analyzed by Fisher's exact probability test (two tailed). $P<0.05$ was considered statistically significant between the frequencies of Recipient Vs Donor for single locus antigens. P=0.00001, statistically significant between the frequencies of Recipient Vs Donor used by χ^2 –test for two locus and three locus haplotypes. Haplotype frequencies were calculated by SPSS version 2.4. Two sided significant values were calculated by Chi Square test.

Results

Out of 2000 live recipients and donors, 825 males and 175 females were recipients, 330 males and 670 females were donors. Majority of recipients were between age group of 21- 40 years and the donors were between age group of 31-50 years.

Antigens with the frequency of 5% or more among recipients and donors for HLA A locus were A*01 :01,A*11:01,A*24:02,A*02:01,A*02:11,A*03:01, A*33:01. The frequency of HLA A*33:01 was high in donors compared to recipients (P value equals 0.0250 - statistically significant), as shown in Table.1.

Table 1. HLA A locus antigen frequencies among recipients and donors

HLA A LOCUS	Recipient			Donor		P Value
	N	%		N	%	
A*02:01	145	7.20%		40	10%	0.185
A*02:11	125	6.20%		32	8%	0.3909
A*02:03	55	2.70%		7	1.75	0.4704
A*02:06	15	0.70%		4	1%	1
A*01:01	375	18.75%		67	16.75%	0.4646
A*11:01	325	16.20%		52	13%	0.1871
A*24:02	310	15.50%		68	17%	0.5936
A*24:07	25	1.20%		NIL	NIL	0.0609
A*33:01	90	4.50%		34	8.50%	0.025
A*33:03	70	3.50%		15	3.75%	1
A*03:01	100	5%		23	5.75%	0.7472

Antigens with the frequency of 5% or more in recipients for HLA-B locus - B*40:06 ,B*51:01,B*52:01,B*35:0 3,B*35:01, B*57:01 , and among donors - B44*03:01,B*57:03, B*15:18, as shown in Table.2.

Table 2. HLA B locus antigen frequencies among recipients and donors

HLA B LOCUS	Recipient		Donor		P Value
	N	%	N	%	
B*40:06	230	11.50%	43	10.75%	0.8101
B*40:01	60	3%	7	1.75	0.3475
B*51:01	225	11.25%	49	12.25%	0.7236
B*51:06	35	1.75%	3	0.75%	0.3375
B*35:03	125	6.25%	21	5.25%	0.6386
B*35:01	125	6.25%	23	5.75%	0.8779
B*52:01	165	8.20%	26	6.50%	0.3977
B*44	145	7.25%	35	8.75%	0.4955
B*44:03:01	75	3.75%	31	7.75%	0.018
B*44:03:02	25	1.25%	NIL	NIL	0.0609
B*44:03:03	20	1%	NIL	nil	0.1231
B*15:18	20	1%	21	5.25%	0.0006
B*15:01	20	1%	8	2%	0.3803
B*15:17	20	1%	6	1.50%	0.7508
B*15:25	10	0.50%	NIL	nil	0.4987
B*57:01	100	5%	6	1.5 %	0.0073
B*57:03	35	1.75%	26	6.5%	0.0008
B*07:02	85	4.20%	20	5%	0.7304

The frequencies of B*44:03(pvalue-0.018), B*15:18(pvalue-0.0006), B*57:03(pvalue-0.0008) were significantly high in donors, while frequency of B*57:01 (pvalue-0.0073) was significantly high among the recipients. Antigens with the frequency of 5% or more in recipients and donors for HLA-DRB1 locus included DRB1*15:01, DRB1*15:02, DRB1*07:01, DRB1*13:01,DRB1*04:03,DRB1*14:04,DRB1*04:03,DRB1*12:02,DRB1*11:01, DRB1*14:04.There were no significant differences in frequencies of HLA DRB1 locus antigens among the recipients and donors as shown in Table.3.

Table 3. HLA- DRB1 locus antigens frequencies among recipients and donors

HLA DRB1 LOCUS	Recipient		Donor		P Value
	N	%	N	%	
DRB1*15:01	205	12.50%	46	11.50%	0.628
DRB1*15:02	195	7.50%	37	9.25%	0.8987
DRB1*07:01	270	13.50%	70	17.50%	0.1047
DRB1*13:01	150	7.50%	29	7.25%	1
DRB1*04:03	140	7%	22	5.50%	0.45
DRB1*04:01	25	1.25%	3	0.70%	0.7238
DRB1*04:05	35	1.75%	7	1.75%	1
DRB1*14:04	125	6.25%	20	5%	0.5272
DRB1*14:01	30	1.50%	5	1.25%	1
DRB1*10:01	95	4.75%	17	4.25%	0.8616
DRB1*12:02	95	4.75%	23	5.75%	0.6251
DRB1*11:01	70	3.50%	21	5.25%	0.2883

Two locus HLA A-B haplotypes A*24:02- B*51:01 and A*01:01- B*52:01 were present among recipients and absent among the donors as shown in Table.4.

Table-4: Most common HLA-A-B haplotypes of renal transplant Recipients & donors

Antigen	Recipient		Donor		P-value
	N	%	N	%	
A*01-B*15	5	6.8	7	10.4	0.041
A*01-B*35	9	12.3	6	9	0.0535
A*01-B*37	12	16.4	6	9	0.0321
A*01-B*40	7	9.6	10	14.9	0.0413
A*01-B*51	8	11	6	9	0.061
A*01-B*52	5	6.8	0	0	0.00001
A*01-B*57	6	8.2	11	16.4	0.0023
A*02-B*07	5	6.5	7	8.4	0.081
A*02-B*51	10	13	9	10.8	0.0625
A*03-B*35	6	30	6	23.1	0.031
A*11-B*35	10	15.4	7	13.5	0.0624
A*11-B*40	12	18.5	6	11.5	0.009
A*24-B*35	10	14.9	5	7.2	0.0026
A*24-B*40	14	20.9	13	18.8	0.091
A*24-B*51	9	13.4	0	0	0.00001

Two locus HLA A-DRB1 haplotypes A*02:01-DRB1*12:02, A*02:01-DRB1*14:04,A*03:01-DRB1*13:01,A*11:01-DRB1*04:03,A*11:01-DRB1*14:04,A*24:02-DRB1*04:01,A*68:01- DRB1*14:01 were present among recipients(Pvalue=0.00001),andA*01:01-DRB1*10:01,A*02:11-DRB1*11:01,A*11:01-DRB1*11:02,A*11:01-DRB1*12:02,A*68:01-DRB1*13:01 were present among donors (P value=0.00001) as shown in Table.5.

Table-5: Most common HLA-A-DR B1 haplotypes of renal transplant Recipients & donors .

Antigen	Recipient		Donor		P-value
	N	%	N	%	
A*01-DRB1*04	45	12.3	6	9	0.042
A*01-DRB1*07	90	24.7	20	29.9	0.037
A*01-DRB1*10	0	0	5	7.5	0.00001
A*01-DRB1*15	30	8.2	8	11.9	0.041
A*02-DRB1*11	0	0	7	8.4	0.00001
A*02-DRB1*12	30	7.8	0	0	0.00001
A*02-DRB1*13	50	13	6	7.2	0.0324
A*02-DRB1*14	25	6.5	0	0	0.00001
A*02-DRB1*15	90	23.4	28	33.7	0.008
A*03-DRB1*13	25	25	0	0	0.00001
A*11-DRB1*04	50	15.4	0	0	0.00001
A*11-DRB1*07	35	10.8	7	13.5	0.0513

Cont... **Table-5: Most common HLA-A-DR B1 haplotypes of renal transplant Recipients & donors .**

A*11-DRB1*11	0	0	7	13.5	0.00001
A*11-DRB1*12	0	0	5	9.6	0.00001
A*11-DRB1*14	30	9.2	0	0	0.00001
A*11-DRB1*15	90	27.7	10	19.2	0.02
A*24-DRB1*04	35	10.4	0	0	0.00001
A*24-DRB1*07	30	9	9	13	0.044
A*24-DRB1*15	85	25.4	15	21.7	0.0461
A*26-DRB1*07	25	33.3	5	31.3	0.059
A*68-DRB1*13	0	0	7	28	0.00001
A*68-DRB1*14	40	42.1	0	0	0.00001

Two locus HLA B-DR haplotypes B*35:03-DRB1*04:03,B*35:01-DRB1*13:01,B*40:06-DRB1*04:03,B*40:06-DRB1*07:01,B*40:01-DRB1*13:01,B*40:06-DRB1*14:04,B*49:01-DRB1*13:02, B*52:01-DRB1*04:03 were present among recipients (P value = 0.00001).Two locus HLA B-DR haplotypes B*13:01-DRB1*07:01,B*44:03-DRB1*15:01, B*51:01-DRB1*04:03, B*51:01-DRB1*07:01 were present among donors (P value = .00001) as shown in Table.6.

Table-6: Most common HLA-B-DR B1 haplotypes of renal transplant Recipients & donors.

Allele	Recipient		Donor		P-value
	N	%	N	%	
B*07-DRB1*13	25	18.5	5	15.6	0.042
B*07-DRB1*15	50	37	10	31.3	0.03
B*08-DRB1*03	25	50	7	77.8	0.0002
B*13-DRB1*07	0	0	6	11.1	0.00001
B*15-DRB1*04	25	17.2	6	14.6	0.052
B*15-DRB1*07	25	17.2	5	12.2	0.037
B*15-DRB1*12	25	17.2	9	22	0.04
B*15-DRB1*15	30	20.7	5	12.2	0.0009
B*35-DRB1*04	25	10	0	0	0.00001
B*35-DRB1*13	25	10	0	0	0.00001
B*37-DRB1*10	25	35.7	5	41.7	0.02
B*40-DRB1*04	35	12.1	0	0	0.00001
B*40-DRB1*07	40	13.8	0	0	0.00001
B*40-DRB1*13	30	10.3	0	0	0.00001
B*40-DRB1*14	30	10.3	0	0	0.00001
B*40-DRB1*15	75	25.9	19	37.3	0.00219
B*44-DRB1*07	50	34.5	17	47.2	0.0022
B*44-DRB1*15	0	0	5	13.9	0.00001
B*49-DRB1*13	35	87.5	0	0	0.00001
B*51-DRB1*04	0	0	7	13.5	0.00001
B*51-DRB1*07	0	0	7	13.5	0.00001
B*51-DRB1*15	50	18.9	8	15.4	0.0523
B*52-DRB1*04	30	18.2	0	0	0.00001
B*52-DRB1*15	55	33.3	11	42.3	0.0059

Three locus HLA A- B-DRB1 haplotypes most common among recipients were A*24:02-B*40:06-DRB1*15:01(P value= 0.00001) and among donors included A*01:01-B*57:01-DRB1*07:01, A*02:11-B*35:03-DRB1*15:01, A*33:01-B*44:03-DRB1*07:01 (P value= 0.00001) . A*02:01-B*51:01-DRB1*15:01 was common among both donors and recipients .

Discussion

It was observed in our study that majority of the donors were females (67.5%) and males outnumbered females in recipients (82.5%), this is in concordance to the study done from various parts of India and Nepal [10,11,12]. Among 2000 live donor and recipient , parents Vs. offspring were 42%, offspring Vs. parents were 7% , spousal donors were 17.5% , siblings were 15.5% ,this is in concordance to study done from North India and Nepal [12,13].

Studies from North India [13] Western India [14], Karnataka, South India [10] and West Central India [15] showed that there was no significant difference of antigens in recipients and donors and in these studies HLA typing was performed by CDC NIH protocol and PCR-SSP method. HLA typing in our study was done by t*he luminex based PCR SSOP method which* enable HLA genotyping on intermediate or in parts high resolution level and *enables a high-throughput typing of HLA antigens* without ambiguity by MatchIT DNA program based on the CWD HLA alleles[16].The HLA antigen frequencies among recipients and donors reported in our study is different from studies done from other parts of India [10,15,17,18,19]. Different subtypes within the same antigens of HLA-A,B,DRB1 loci were also documented in our study and this was possible by Luminex PCR-SSO typing .*From our results we can infer that B*57:01 antigen may be a risk factor for renal failure and* HLA A*33:01, B*44:03, B*15:18, B*57:03 antigens may have protective influence on the kidney. Two locus haplotypes A*24:02-B*51:01, A*01:01- B*52:01, A*68:01-DRB1*14:01, B4*9:01-DRB1*13:02 were significantly high among recipients and absent in donors indicating that this haplotype may be a risk factor for renal failure, while A*68:01-DRB1*13:01,B*44:03-DRB1*15:01 were significantly high among donors indicating that this haplotype may have protective influence on kidney,these haplotypes are not documented from other parts of India [10,13,15,16,17]. Three locus haplotypes among donors was A*02:01-B*51:01-DRB1*15:01 and this haplotype is not reported from other parts of India ,followed by A*33:01:01-B*44:03:02-DRB1*07:01:01 which is similar to the study done from North India [17] and A*02:11-B*35:01-DRB1*15:01,from South India[21] , these haplotypes may have protective influence on kidney. Three locus haplotype reported among the recipients in our study was A*01:01-B*57:01-DRB1*07:01, this haplotype is not reported from other parts of India.The present study is the first investigation of HLA typing by PCR-SSOP method, for live related renal transplant recipients and donors from Telangana state of South India.

Conclusion

The knowledge of HLA allele and haplotype frequencies will help in understanding the Indian population genetics and help the organ transplant team to find suitable matched donors for transplant recipients.

Conflict of Interest: No

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