HLA FREQUENCIES IN PAKISTANI POPULATION

Pages with reference to book, From 294 To 298

Abdul Hannan, Masood Anwar, Manzoor Ahmad (Armed Forces Institute of Pathology, Rawalpindi.)
Tahir Aziz Ahmed (Armed Forces Institute of Pathology,Rawalpindi)
Lubna Zafar, Farhat Rizvi (Armed Forces Institute of Pathology,Rawalpindi.)

ABSTRACT

HLA frequencies on 1231 subjects from within the country, using methods employed by National Institute of Health, USA is reported. Pakistani population appears to be a mixture of an indigenous population with others particularly Orientals and Negroids. Relationship with Caucasians is not convincing (JPMA 40 : 294, 1990).

INTRODUCTION

The HLA system is known to be the most polymorphic genetic system found in humans. It plays a vital role in transplantation, immune responses, disease associations, paternity testing and anthropological studies. Accordingly, a number of studies have been carried out on different populations and ethnic groups to collect basic data on their antigen and gene frequencies. Studies have been carried out on HLA frequencies in Pakistanis settled in other countries[1-3]. Not only the number of subjects tested in such studies are limited, the purity of their ethnic origin is also suspect. A study of HLA system has, so far, not been reported from within Pakistan. This study presents the HLA frequencies in Pakistani population.

MATERIAL AND METHODS

A total of 1231 subjects (920 males and 311 females) were tested for HLA-A and B loci. Only 220 subjects (159 males and 61 females) were tested for HLA-C locus. The class II antigens (HLA-DR and HLA-DQ) were tested in 1166 subjects (862 males and 304 females). The age of subjects ranged from 3 years to 65 years. All the subjects were either prospective recipients of renal/bone marrow transplant or prospective donors. Donors included both related and non-related donors. Details are shown in Table-I.

TABLE I. Details of subjects tested for HLA antigens.

	A&B Loci		C Locus		DR & DQ Loci	
	Male	Female	Male	Female	Male	Female
Patients	245	61	35	8	236	61
Donors	675	250	124	53	626	243
Total	920	311	159	61	862	304
Total	1231		220		1166	

Venous blood samples were collected in heparinized vacuutainer tubes and were processed within 12 hours. The lymphocytes were separated on Ficoll-Hypaque density gradient by centrifugation in a

refrigerated centrifuge. The B cells were separated by adhesion to nylon wool in a column[4]. The HLA antigens were tested by the two stage NIH microlymphocytotoxicity assay using Terasaki plates[4]. Antisera were obtained commercially from Biotest, Pel-Freeze and Behring Laboratories. The relative antigen frequencies were determined and gene frequencies were estimated from antigen frequencies using the formula p = 1-/1-f where p denotes the gene frequency and f denotes the antigen frequency[4]. AB haplotypes were then calculated from these frequencies.

RESULTS

The results are shown and compared with the antigen and gene frequencies of other population groups in Tables II to VI.

TABLE II. Comparison of HLA-A antigen and gene frequencies in different population groups.

af = Antigen frequency, gf = Gene frequency

	PAKISTANIS		CAUCASIANS		ORIENTALS		NEGROIDS	
HLA	af	gf	af	gf	af	gf	af	gf
A1	.248	.133	.264	.142	.020	.010	.155	.081
A2	.314	.172	.494	.289	.483	.281	.319	.175
A3	.132	.068	.247	.132	.030	.015	.130	.067
A9	.199	.105	.220	.117	.531	.315	.240	.128
A10	.178	.093	.115	.059	.154	.080	.188	.099
A11	.232	.124	.122	.063	.220	.117	.038	.019
A23 (A9)	.006	.003	.028	.014	.002	.001	.154	.080
A24 (A9)	.009	.005	.195	.103	.529	.314	.094	.048
A26 (A10)	.006	.003	.063	.032	.139	.072	.088	.045
A28	.085	.043	.092	.047	.042	.021	.108	.099
A29 (AW19)	.022	.011	.057	.029	.008	.004	.096	.049
A30 (AW19)	.037	.019	.069	.035	.045	.023	.208	.110
A31 (AW19)	.032	.016	.057	.029	.101	.052	.032	.016
A32 (AW19)	.020	.010	.076	.039	.008	.004	.045	.023
AW33 (AW19)	.058	.029	.028	.014	.116	.060	.076	.039
AW36	.008	.004	.002	.001	.002	.001	.063	.032
AX	.347	.192		.004		.017		.050

TABLE III. Comparison of HLA-B antigen and gene frequencies in different population group.

HLA	PAKITANIS		CAUCASIANS		ORIENTALS		NEGROIDS	
	af	gf	af	gf	af	gf	af	gf
B5	.027	.144	.157	.082	.279	.151	.049	.025
B7	.086	.044	.217	.115	.092	.047	.227	.121
B8	.175	.092	.183	.096	.004	.002	.107	.055
B12	.096	.049	.238	.127	.118	.061	.190	.100
B13	.064	.033	.057	.029	.075	.038	.032	.016
B14	.007	.004	.073	.037	.004	.002	.057	.029
B15	.024	.012	.131	.068	.183	.096	.088	.045
B16	.003	.002	.088	.045	.022	.011	.032	.016
B17	.146	.076	.057	.029	.038	.019	.057	.029
B18	.015	.008	.107	.055	.006	.003	.082	.042
B21	.042	.021	.057	.029	.012	.006	.057	.029
BW22	.026	.013	.055	.028	.195	.103	.006	.003
B27	.063	.032	.067	.034	.032	.016	.038	.019
B35	.229	.122	.199	.105	.194	.102	.137	.071
B37	.008	.004	.032	.016	.012	.006	.026	.013
B39 (B16)	.008	.004	.004	.002	.008	.004	.0	.0
B40	.202	.107	.115	.059	.331	.182	.075	.038
B44 (B12)	.021	.011	.231	.123	.116	.060	.148	.077
BW46	.001	.001	.002	.001	.071	.036	.0	.0
B49 (B21)*	.074	.038	.036	.018	.006	.003	.045	.023
B51 (B5)	.002	.001	.120	.062	.150	.078	.038	.019
BW52 (B5)*	.015	.008	.004	.002	.141	.073	.012	.006
BW55 (B22)	.005	.003	.032	.016	.042	.021	.0	.0
BW60 (B40)	.024	.012	.075	.038	.126	.065	.045	.023
BW62 (B40)	.007	.004	.118	.061	.183	.096	.051	.026
BW63 (B15)	.019	.009	.014	.007	.0	.0	.038	.019
BW73	.049	.025	.002	.001	.004	.002	.0	.0
BX	.323	.177		.004		.016		.013

TABLE IV. Comparison of HLA-C Antigen and Gene frequencies in different populatin groups.

HLA	PAKISTANIS		CAUCASIANS		ORIENTALS		NEGROIDS	
	af	gf	af	gf	af	gf	af	gf
CW1	.054	.027	.065	.033	.299	.163	.020	.010
CW2	.170	.089	.080	.041	.020	.010	.224	.119
CW3	.265	.143	.236	.126	.471	.273	.159	.083
CW4	.489	.285	.219	.116	.103	.053	.260	.140
CW5	.072	.037	.133	.069	.012	.006	.059	.030
CW6	.094	.048	.165	.086	.075	.038	.241	.129
CW7	.126	.065	.427	.243	.227	.121	.424	.241
CX	.0	.482	.0	.249	.0	.333	.0	.213

TABLE V. Comarison of HLA-DR antigen and gene frequencies in different population groups.

HLA	PAKISTANIS		CAUCASIANS		ORIENTALS		NEGROIDS	
	af	gf	af	gf	af	gf	af	gf
DR1	.164	.086	.181	.095	.098	.050	.099	.051
DR2	.335	.185	.291	.158	.279	.151	.279	.151
DR3	.294	.156	.226	.120	.036	.018	.276	.149
DR4	.123	.064	.238	.127	.388	.218	.146	.076
DR5	.290	.157	.266	.143	.211	.112	.358	.199
DRW6	.016	.018	.211	.112	.185	.097	.269	.145
DR7	.147	.076	.226	.120	.057	.029	.247	.132
DRW8	.024	.012	.059	.030	.141	.073	.016	.008
DRW9	.010	.005	.016	.008	.217	.115	.020	.015
DRW10	.043	.022	.016	.008	.010	.005	.045	.023
DRW11 (DR5)	.013	.007	.231	.123	.078	.040	.303	.165
DRW12 (DR5)	.019	.010	.004	.002	.139	.072	.067	.034
DRW14 (DRW6)	.004	.002	.113	.058	.131	.068	.203	.107
DRX		.372		.79		.132		.053

TABLE VI. Comparison of HLA-DQ antigen and gene frequencies in differnt population groups.

HLA	PAKISTANIS		CAUCASIANS		ORIENTALS		NEGROIDS	
	af	gf	af	gf	af	gf	af	gf
DQW1	.245	.131	.542	.323	.513	.302	.641	.401
DQW2	.003	.002	.329	.181	.098	.050	.409	.231
DQW3	.026	.013	.412	.233	.547	.327	.431	.246
DQX		.418		.263		.321		.122

The data for comparison is obtained from the Proceedings of Histocompatibility workshop 1984[5]. The original values were expressed in percentages but we have converted these to decimal figures for comparison. Since in majority of subjects only HLA-A and HLA-B antigens were tested, therefore haplotypes are determined only for these antigens. These are shown in Table-VII.

TABLE VII. HLA A and B haplotypes true and expected frequencies

Upper figure = Observed frequency. Lower figure = Expected frequency. For haplotype read A against B.

Antigen	A1	A2	A3	A9	A10	All	A23	A24	A26	A28	A29	A30	A31	A32	AW33	AW36
B5	.018	.033	.016	.020	.016	.023	.001	.001	0	.017	.001	.003	.005	.003	.003	.001
	.019	.025	.010	.015	.013	.018	0	.001	0	.006	.002	.003	.002	.001	.004	.001
B7	.007	.008	.007	.006	.003	.004	0	.001	0	.007	.007	.001	.002	.001	.002	0
	.006	.008	.003	.005	.004	.005	0	0	0	.002	.001	.001	.001	0	.001	0
B8	.013	.015	.008	.012	.035	.011	0	.001	.001	.007	.001	.001	.003	.001	.003	0
	.012	.016	.006	.010	.009	.011	0	.001	0	.004	.001	.002	.002	.001	.003	0
B12	.009	.012	.004	.007	.005	.007	0	0	0	.003	.001	.001	.001	.001	.005	0
	.007	.008	.003	.005	.005	.006	0	0	0	.002	.001	.001	.001	.001	.001	0
B13	.005	.007	.002	.004	.002	.005	0	0	0	.003	.001	.005	0	0	.001	0
	.004	.006	.002	.004	.003	.004	0	0	0	.001	0	.001	0	0	.001	0
B14	.001	0	.001	.001	.002	0	0	0	0	0	0	0	0	0	0	0
	.001	.001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B15	.001	.004	.001	.002	.001	.003	0	0	0	.001	0	0	0	0	.001	.001
	.002	.002	.001	.001	.001	.002	0	0	0	.001	0	0	0	0	0	0
B16	0	.001	0	0	0	0	0	0	0	0	0	.001	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B17	.025	.014	.006	.006	.008	.007	.001	0	0	.008	.001	.002	.002	.001	.006	0
	.010	.013	.005	.008	.007	.009	0	0	0	.003	0	.001	.001	0	.002	0
B18	.001	.001	0	.002	.001	.001	0	0	0	.001	0	0	0	0	0	0
	.001	.001	0	.001	0	.001	0	0	0	0	0	0	0	0	0	0
B21	.001	.008	.002	.002	.003	.004	.001	0	0	.001	0	0	.001	.001	.001	0
	.003	.004	.001	.002	.002	.003	0	0	0	.001	0	0	0	0	0	0
BW22	.004	.002	.001	.003	.001	.003	0	0	0	.002	0	0	0	0	.001	0
	.002	.002	.001	.001	.001	.002	0	0	0	.001	0	0	0	0	0	0
B27	.007	.007	.002	.004	.005	.005	0	0	0	.002	.001	0	.001	0	.001	0
	.004	.006	.002	.003	.003	.004	0	0	0	.001	0	0	.001	0	0	0
B35	.019	.024	.015	.017	.008	.023	0	.001	.001	.013	.001	.003	.003	.002	.005	.001
	.016	.021	.008	.013	.011	.015	0	.001	0	.005	.001	.002	.002	.001	.004	.001
B37	.003	.001	0	0	0	.001	0	0	0	0	0	0	0	0	0	0
	.001	.001	0	0	0	.001	0	0	0	0	0	0	0	0	0	0
B39	0	.001	0	0	.001	.001	0	0	0	0	0	.001	.001	0	0	0
	.001	.001	0	0	0	.001	0	0	0	0	0	0	0	0	0	0
B40	.017	.027	.007	.020	.009	.020	.001	.002	.001	.010	0	0	.001	.001	.003	.001
	.014	.018	.007	.011	.010	.013	0	.001	0	.005	.001	.001	.001	.001	.003	0
B44	.001	.003	.001	.002	0	.001	0	0	0	.002	0	0	0	0	.002	0
	.001	.002	.001	.001	.001	.001	0	0	0	.001	0	0	0	0	0	0
BW46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B49	.006	.007	.004	.006	.006	.006	.001	0	0	.003	.001	.001	.001	.001	.001	0
	.005	.007	.003	.004	.004	.005	0	0	0	.002	0	.001	.001	0	.001	0
B51	.001	.001	.001	.002	0	0	0	0	0	.001	0	0	0	0	.001	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BW52	0	0	0	.001	0	0	0	.001	0	0	0	0	0	0	0	0
	.001	.002	.001	.001	.001	.001	0	0	0	0	0	0	0	0	0	0
BW55	.003	.003	.002	.001	0	.003	0	0	0	.001	0	0	0	.001	0	.001
	0	.001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BW60	0	.001	0	0	.001	.001	0	0	0	0	0	0	0	0	0	0
	.002	.002	.001	.001	.001	.001	0	0	0	0	0	0	0	0	0	0
BW62	0	.001	0	0	.001	.001	0	0	0	0	0	0	0	0	0	0
	.001	.001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BW63	.005	.002	.001	.002	0	.001	0	0	0	0	0	0	0	0	0	0
	.001	.002	.001	.001	.001	.001	0	0	0	0	0	0	0	0	0	0
BW73	.003	.004	.004	.001	.005	.003	0	0	0	.004	0	.001	0	0	.001	0

DISCUSSION

The existence of histocompatibility antigens was first proposed by Landoteiner in 1931 in his Nobel lecture[6]. Gorer, in his pioneering work on mice, proved the existence of such a system[6]. The work carried out by Dausset, van Rood and Payne led to the formation of a base for a comparable system in man, and the system was named “the human leucocyte antigen (HLA) system” [6].Its development to

present stage would not have been possible without international collaborative efforts. A system of workshops was initiated by D.B. Amos in 1964 (which still continues). It added new dimensions to this very important and most polymorphic antigen system[6]. The fourth and fifth International Histocompatibility Workshops, organized in 1970 and 1972, assembled data from over 100 different populations and found that the frequencies of HLA antigens varied markedly from one ethnic group to another[7]. Such observations have helped to gain an insight into the origins and associations of various populations. Earlier studies in HLA system in Pakistanis have tried to place this population at its place in anthropological map of the world[2,3]. There has been a general opinion that Pakistani population is more near Caucasians. But if we compare first 5 most common antigens of each locus in different population groups, a different picture emerges.

TABLE VIII. Comparison of most common antigens of Pakistani population with other population groups.

HLA	PAKISTANIS		CAUCASIANS		ORIENTALS		NEGROIDS	
	af	gf	af	gf	af	gf	af	gf
Class I								
A 1	.248	.133	.264	.142	–	–	–	–
.A 10	.178	.093	–	–	.154	.080	.188	.099
A 11	.232	.124	–	–	.220	.117	–	–
B5	.267	.144	-	-	.279	.151	-	-
B 8	.175	.092	–	–	–	–	.107	.055
B 17	.146	.076	–	–	–	–	–	–
B 40	.202	.107	–	–	.331	.182	–	–
CW2	.170	.089	–	–	–	–	.224	.119
Class II								
DR1	.164	.086	–	–	–	–	–	–
DR2	.335	.185	–	–	.279	.151	.279	.151
DR3	.294	.156	.226	.120	–	–	.276	.149
DR7	.147	.076	.226	.120	–	–	–	–

Table VIII shows antigen and gene frequencies for these after excluding antigens which are shared by all population groups as first five most common antigens. Detailed comparison of all antigens is shown in tables II to VI. Our findings suggest that Pakistanis are closer to Orientals and Negroids, rather than Caucasians. Only one class I Antigen (Ai) is shared by Pakistanis and Caucasians as one of first five most common antigen groups (1/5) while 4/5 and 3/5 class I antigens are shared with Orientals and Negroids, respectively. One antigen B17 appears to be most prevalent in Pakistanis only. Among class II antigens 2/5 are shared with Caucasians and Negroids while 1/5 is shared with Orientals. One antigen (DRi) appears to be most prevalent again only in Pakistanis. These figures suggest mixing of an indigenous population mostly with Negroids and orientals but also with Caucasians. A detailed study of HLA system in various castes and tribes may produce very interesting results. In table-VII at many points the true frequency of AB haplotype is much higher than expected. This is called linkage disequilibrium. This is particularly evident for A28 B5, Aio, B8 and A1, B17. Most likely this is because of presence of greater number of related persons in our study. Proportion of blanks (x) is also higher as compared to other studies. This may be due to absence of about 18 antisera from our plates. This is only the beginning of studying HLA system in Pakistan. More studies are required to establish concrete basis of this system for our population.

REFERENCES

1. Singal, D.P. The distribution on HLA Leucocyte Antigens in In. dians, in Histocompatibility testing. Copenhagen, Munksgaard, 1972; p.179.
2. Harris, R., Wentzel, J., Carroll, C.A. and Jennison, R.F. HLA frequencies in West Pakistanis (Punjabi) in the United Kingdom, in histocompatibility testing. Copenhagen, Munksgaard, 1972, p.163.

3. Solheim, B.G., Bratlie, A. and Thorsby, E. A study of the HLA system in a West Pakistan population, in histocompatibility testing. Copenhagen, Munksgaard, 1972, p.171.
4. Staff of NIH, Transplantation and Immunology Branch. NIAID manual of tissue typing techniques 1979-1980. U.S. Deptt. of Health and Human Services, NIH Publication No. 85-545; 1980, p.39.
5. Albert, E.D., Baur, M.P. and Mayr, W.R. Population analyses on the basis of deduced haplotypes from random families, in histocom patibility testing. New York., Springer — Verlag, 1984.
6. Bodmer,W.RTheHLAsystem: Introduction. Br. Med. Bull., 1978;34:213.
7. Ting, A. and Morris, P. J. The relationship of six ethnic groups (Chinese, Malays, Indians, New Guinea highlands and coastal natives, Australian, Caucasians). Based on the HL-A System, in histocompatibility testing. Copenhagen, Munksgaard, 1972, p.275.