

HLA Antigens in Nadars a Native Dravidian Caste Group of Tamil Nadu, South India

B. Sridharan¹ and U. Shankarkumar²

1. Department of Medicine, Government Rajaji Hospital, Madurai 625 001, Tamilnadu, India

2. HLA Department, Institute of Immunohaematology (ICMR), 13th Floor, NMS Bldg., K.E.M. Hospital, Parel, Mumbai 400 012, Maharashtra, India

KEYWORDS HLA; Nadar caste; Dravidian; genetic diversity

ABSTRACT One hundred and thirty seven unrelated Nadar individuals residing in Tamil Nadu, (South India) were studied for HLA A, B, C and DR locus antigen profiles. The allele frequencies of HLA A9, B5, Cw4, Cw7, DR2 and DR6 were increased while frequencies of HLA A10, B8, B16, B46, B78, Cw5, Cw9 and DR9 were decreased in the Nadars. The gene frequencies of HLA A1, A3, A28, B5, B17, B37, Cw4, Cw5, DR1, DR6 and DR10 were increased while that of A2, A19, B16, B46, Cw3, DR5 and DR9 were decreased when compared with gene frequencies of other Asian populations reported. Two Locus haplotype analyses revealed that A11-B5, A1-B17, A3-B44, A24-B51, B5-Cw9, B44-Cw1, DR4-DQ2 and DR6-DQ1 were significant haplotypes among the positive linkage disequilibrium haplotypes. Where as A1-B5, B5-Cw1 and DR1-DQ2 were significant haplotypes among the negative linkage disequilibrium haplotypes. The haplotypes A3-B44, A24-B51, B44-Cw1 and DR6-DQ1 observed in Nadars were unique when compared to other Indian populations reported in literature. The observed antigen frequencies and linkage disequilibrium in Nadars suggest the influence of genetic drift caused by selection, geography and culture with a lesser degree of admixture. Further the study reveals that the Nadar population of India cannot be considered as a single panmictic population with reference to genetic characteristics, which may have a clinical relevance in unrelated donor selection for allogeneic Bone marrow transplantation in India.

INTRODUCTION

India is thought to have been one the site of earliest human settlements. It is home to scores of cults and religions, including all the major world religion. All religions not only coexist, but also flourish in India due to the eclectic nature of Hinduism. Hindus worship 330 million Gods. The polytheistic eclecticism lends to Hinduism a capacity to coexist with other religions. The region has been subjected to successive waves of immigration and invasions from the Middle East, Central Asia and Mongolia, contributing to the present day gene pool (Bhasin et al. 1994; Bhasin and Walter 2001). Risely (1969) was the first to study the "racial types" in Indian population. Malhotra (1978) described the Western and Southern Indian population as Australoid or Proto-Australoid elements. The present-day population is a panorama of social, cultural and ethnically distinct (Papiha 1996). The population exhibits not only a wide variety of ethnic but also great cultural and linguistic diversity. The social structure is governed by a large number of religious groups. Eighty two percent of Indian population is Hindus, while

the other minor religions include Christians, Sikhs, Buddhists, Jains and Muslims. Numerous endogamous ethnic groups delineate within each linguistic or religious group based on biological and socio-cultural characteristics (Haimendorf 1948). Tamil Nadu is the southern most part of the peninsular Southern India, lying between 77° – 80° E and 8° – 13° N and it has one of the most populous people majority speaking Dravidian language. Dravidians of South India is more a culture and a unique social institution, with a unique linguistic family subdivided into many gene pools, differing in their origin, migration and settlement (Menon 1996; Pitchappan 2002). Nadars a Tamil speaking Dravidian caste are concentrated in Chennai, Madurai, Theni, Virudhunagar, Thirunelveli, Kanyakumari and Nagercoil districts of Tamil Nadu. In terms of occupation they were regarded initially as "toddy-tappers" while latter adapted into, merchants, professionals and agriculturists. They are considered as one of the earliest inhabitants of South India and are believed to have their origin in South known as "Komari Land" which is the originator of great Mediterranean culture extending to Africa, Australia and Middle east

countries (Hardgrave 1969).

Previous studies on caste groups from Tamil Nadu brought out their genetic differences and are indeed as high as that of two major ethnic groups of the World (Pitchappan 1988; Rajasekar et al. 1987; Balakrishnan et al. 1996). Further various populations and caste of South India studied revealed HLA antigen disease associations as well (Brahmajothi et al. 1991; Ravikumar et al. 1999; Shanmugalakshmi et al. 2003). Here we present the HLA polymorphism of Nadars living in Virudhunagar District of Tamil Nadu and compared them with results of selected populations of India and World.

MATERIALS AND METHODS

Blood samples from random 137 healthy unrelated Nadars from South India were studied for HLA -A, -B, -C and -DR Locus antigen profiles. Ten to fifteen milliliters of venous blood (in heparin 50 IU/ml) was collected in a sterile tube from each individual after recording their population specific details, informed consent and genealogy. The lymphocytes were isolated by density gradient centrifugation on Histopaque (Boyum 1968). HLA A, B, C and DR locus antigens were identified by NIH two – stage Microlymphocytotoxicity assay (Terasaki and McClelland 1964) using T cells for class I typing and B cells isolated by a miniature nylon wool column for class II with a longer incubation period (Manikasundari et al. 1984). A total of 190 antiserum were used for defining 17 specificities for HLA A locus, 29 for HLA B locus, 8 for HLA C locus and 10 for HLA DR locus antigens. The antisera were commercial (Biotest, Germany; Behring, Germany; Pelfreez, USA) as well as indigenous (Shankarkumar et al 1998) in origin. The typing tray included a minimum of three antisera for each supertypic specificity. The phenotype frequency (PF), genotype frequency (GF), standard error of gene frequency (SEGF), haplotype frequency (HF), Co efficient of linkage disequilibrium (Delta) and “ t ” values were calculated following the method described earlier (Shankarkumar et al. 2001).

RESULTS

The results on HLA A, B, C and DR allele frequencies in 137 unrelated healthy Nadars are presented in Table 1. The allele frequencies of HLA A9, B5, Cw4, Cw7, DR2 and DR6 were

increased while frequencies of HLA A10, B8, B16, B46, B78, Cw5, Cw9 and DR9 were decreased in the Nadars. The comparison of HLA A, B, C and DR gene frequencies in Nadars with different Asian populations (Imanshi et al. 1992) are presented in Table 2. The gene frequencies of HLA A1, A3, A28, B5, B17, B37, Cw4, Cw5, DR1,

Table 1: HLA A, B, C and DR antigens in Nadar caste group from Tamil Nadu, South India.

HLA antigens N = 137	N+	%AF
A1	51	18.61
A2	34	12.40
A3	15	5.47
A9	55	20.07
A10	6	2.18
A11	40	14.59
A19	28	9.85
A23	1	0.36
A24	18	6.56
A25	0	0.00
A26	1	0.36
A28	13	4.74
A29	1	0.36
A30	0	0.00
A31	8	2.91
A32	2	0.72
A33	4	1.45
A34	1	0.36
A68	3	1.09
A69	2	0.72
B5	56	20.43
B7	26	9.48
B8	1	0.36
B12	14	5.10
B13	12	4.37
B14	0	0.00
B15	11	4.01
B16	3	1.09
B17	26	9.48
B18	0	0.00
B21	2	0.72
B22	23	8.39
B27	10	3.64
B35	22	8.02
B37	9	3.28
B38	0	0.00
B39	0	0.00
B40	22	8.02
B44	5	1.82
B45	1	0.36
B46	1	0.36
B49	1	0.36
B50	0	0.00
B51	19	6.93
B52	11	4.01
B54	0	0.00
B55	1	0.36
B56	4	1.45
B57	6	2.18
B61	4	1.45

Table 1: Contd....

<i>HLA antigens</i> <i>N = 137</i>	<i>N+</i>	<i>%AF</i>
B62	5	1.82
B63	0	0.00
B78	2	0.72
N = 33		
Cw1	4	6.06
Cw2	0	0.00
Cw3	4	6.06
Cw4	14	21.21
Cw5	2	3.03
Cw6	7	10.60
Cw7	14	21.21
N = 105		
DR1	17	8.09
DR2	30	14.28
DR3	18	8.57
DR4	23	10.95
DR5	15	7.14
DR6	31	14.76
DR7	18	8.57
DR8	17	8.09
DR9	9	4.28
DR10	15	7.14

N+ = Number positive for the allele, % AF = Percentage Allele Frequency

DR6 and DR10 were increased while that of A2, A19, B16, B46, Cw3, DR5 and DR9 were decreased when compared with gene frequencies of other Asian populations reported. The table 3 present the observed two-locus haplotype frequencies and linkage disequilibrium of Nadars. It is interesting to note that haplotypes A1-B17, A24-B51, B5-Cw9, B44-Cw1 and DR6-DQ1 had high delta and significant 't' values among positive linkage disequilibrium while A1-B5, B5-Cw1 and DR1-DQ2 had high delta and 't' values among negative linkage disequilibrium haplotypes.

DISCUSSION

Theoretically high polymorphism of a gene can occur due to mutation rate, selection, genetic hitchhiking or a combination of all the three (Kaufman 1996). Studies of various Indian populations using PCR based typing have revealed the extent of allelic diversity in HLA loci (Shankarkumar et al. 2003). Earlier population studies have indicated that there are many alleles

Table 2: Gene frequencies (in percentage) of Nadars compared with other Asian populations

<i>HLA</i>	<i>Nadars</i> <i>(N = 137)</i>	<i>Japanese¹</i> <i>(N = 1023)</i>	<i>Thais¹</i> <i>(N = 242)</i>	<i>Vietnamese¹</i> <i>(N = 149)</i>	<i>Singapore Chinese¹</i> <i>(N = 73)</i>
A1	20.80	0.70	2.30	3.70	0.70
A2	13.30	24.40	25.50	25.90	37.00
A3	5.60	0.60	1.50	1.70	0.70
A9	22.60	35.10	14.60	13.80	16.40
A23	0.40	0.00	0.00	0.30	0.00
A24	6.80	14.60	14.60	13.50	16.40
A10	2.20	3.60	3.60	2.10	4.80
A25	0.00	0.40	0.40	0.00	0.00
A26	0.40	1.90	1.90	2.10	0.70
A34	NT	1.30	0.00	4.10	
A66	NT	NT	NT	NT	NT
A11	15.90	10.40	32.50	26.30	26.00
A19	10.40	16.10	17.20	22.00	13.70
A29	0.40	0.00	0.60	8.80	2.10
A30	0.00	0.40	1.10	3.00	0.00
A31	3.00	8.00	1.70	2.30	3.40
A32	0.70	0.00	0.20	0.00	0.00
A33	1.50	7.70	13.60	7.90	8.20
A74	NT	NT	NT	NT	NT
A28	4.90	0.00	0.80	0.70	0.70
A68	1.10	NT	NT	NT	NT
A69	0.70	NT	NT	NT	NT
A36	0.00	0.00	0.20	0.00	0.00
A43	0.00	0.00	0.40	0.00	0.00
A80/A-	12.00	1.70	1.50	3.50	0.00
B5	23.10	20.00	9.50	2.30	5.80
B51	7.20	9.30	6.40	1.30	4.10
B52	4.10	10.70	3.10	1.00	0.70
B7	10.00	5.00	2.70	12.20	2.10

Table 2: Contd....

HLA	Nadars (N = 137)	Japanese ¹ (N = 1023)	Thais ¹ (N = 242)	Vietnamese ¹ (N = 149)	Singapore Chinese ¹ (N = 73)
B8	0.40	0.00	0.20	0.30	0.70
B12	5.20	7.40	5.40	4.10	0.70
B44	1.80	7.40	5.40	3.40	0.70
B45	0.40	0.00	0.00	0.70	0.00
B13	4.50	1.80	9.30	7.40	6.80
B14	0.00	0.10	0.40	0.70	0.00
B64	NT	NT	NT	NT	NT
B65	NT	NT	NT	NT	NT
B15	4.10	9.40	14.10	20.00	14.10
B62	1.80	8.30	5.00	10.10	4.10
B63	0.00	0.00	0.40	1.10	7.90
B75	NT	1.10	8.30	6.70	0.00
B76	NT	0.00	0.00	2.10	2.10
B77	NT	0.00	0.40	0.00	0.00
B16	1.10	4.80	5.20	6.90	7.00
B38	NT	0.30	3.50	4.20	4.10
B39	NT	4.50	1.70	2.70	2.90
B17	10.00	0.70	8.10	5.70	7.20
B57	2.20	0.00	5.20	2.70	0.00
B58	0.00	0.70	2.90	3.00	7.20
B18	0.00	0.00	1.30	0.00	2.50
B21	0.70	0.00	0.00	0.30	0.00
B49	0.40	0.00	0.00	0.00	0.00
B50	0.00	0.00	0.00	0.30	0.00
B22	8.80	10.80	5.50	4.40	6.40
B54	0.00	6.30	0.60	1.70	2.90
B55	0.40	2.90	2.50	1.00	1.40
B56	1.50	1.60	1.40	1.70	2.10
B27	3.70	0.40	6.00	3.20	3.70
B35	8.40	8.10	2.50	5.00	2.10
B37	3.30	0.70	1.40	0.70	0.00
B40	8.40	16.30	12.60	4.90	13.50
B60	NT	5.60	8.30	3.20	11.40
B61	NT	10.70	4.30	1.70	2.10
B41	NT	0.00	0.00	0.30	0.00
B42	NT	0.00	0.20	0.00	0.00
B46	0.40	4.40	14.00	13.20	15.10
B47	NT	0.10	0.00	0.00	1.50
B48	NT	3.20	1.00	1.30	6.20
B53	NT	0.00	0.00	0.00	0.00
B59	NT	1.90	0.00	0.00	0.00
B67	NT	1.50	0.00	0.00	0.70
B70	NT	1.60	0.00	0.70	0.70
B71	NT	NT	NT	NT	NT
B72	NT	NT	NT	NT	NT
B73	NT	0.00	0.00	0.30	0.00
B78	0.70	0.00	0.00	0.00	0.00
B81	NT	NT	NT	NT	NT
Cw1	6.30	11.80	3.30	3.40	11.80
Cw2	0.00	0.10	2.70	0.00	2.10
Cw3	6.30	22.70	21.10	10.00	36.30
Cw4	24.10	4.20	3.60	7.30	4.80
Cw5	3.10	0.40	0.60	1.00	0.00
Cw6	11.20	1.00	7.20	6.90	6.20
Cw7	24.10	15.30	25.50	18.80	18.60
	N= 105	N=898	N= 238	N= 142	N= 77
DR1	8.50	5.50	0.20	1.40	0.60
DR2	15.50	18.20	25.10	6.60	12.90
DR3	9.00	0.20	4.80	4.00	5.20
DR4	11.60	22.80	9.00	8.70	19.50

Table 2: Contd....

HLA	Nadars (N = 137)	Japanese ¹ (N = 1023)	Thais ¹ (N = 242)	Vietnamese ¹ (N = 149)	Singapore Chinese ¹ (N = 73)
DR5	7.40	10.20	17.90	31.80	27.20
DR6	16.00	13.30	10.70	9.80	4.50
DR7	9.00	0.40	8.30	5.80	1.90
DR8	8.50	13.30	4.00	6.70	6.50
DR9	4.40	13.00	11.90	11.50	16.90
DR10	7.40	0.60	2.50	5.70	1.30

1. 11th International Histocompatibility Workshop and Conference 1991

NT = Not Tested, NR = Not Reported

Table 3: Haplotype frequencies and significant Linkage disequilibrium identified in Nadar caste group from South India

Haplotype	HF (%)	LD(%)	t value ^a	Chi ²
<i>Positive Linkage Disequilibrium:</i>				
A11-B5	5.76	2.17	2.12	4.01
A1-B17	5.24	3.16	3.72	14.98
A3-B44	1.45	1.35	2.82	43.67
A24-B51	9.20	4.08	3.58	11.25
B5-Cw9	4.63	2.79	3.63	14.56
B44-Cw1	8.15	3.77	3.63	12.35
DR4-DQ2	3.12	1.83	3.15	11.25
DR6-DQ1	6.28	2.29	2.67	6.40
<i>Negative Linkage Disequilibrium:</i>				
A1-B5	0.08	-4.62	-3.75	15.51
B5-Cw1	0.05	-4.15	-3.81	15.5
DR1-DQ1	0.26	-4.49	-4.39	21.36

HF = Haplotype frequency per 100.

Delta = Linkage disequilibrium per 100.

\$ = significant Negative Linkage disequilibrium.

a = t value > 2 indicates positive for Delta.

and haplotypes that appear to be specific for a given population group. Indigenous populations or caste groups show a very restricted diversity of alleles at a particular HLA loci consistent within a population (Trachtenberg 1995). Moreover specific alleles found uniquely in a particular indigenous group may have been generated by point mutation or gene conversion from the ancestral allele after the group separated from the other groups. Multiple polymorphic alleles in a population are maintained at appreciable frequencies due to either overdominance (heterozygous advantage), frequency dependent selection or other selective force (Apple and Erlich 1996). Both selective forces and a high rate of germline diversification are involved in the evolution of HLA allelic diversity. A newly arisen favorable variant allele might co-exist with the parental allele rather than replacing it when selective forces favoring diversity is operating. Recently novel alleles in the Indian populations

have been reported for HLA A2, A19, B27 and DR2 alleles (Shankarkumar et al. 2002a, b; Mehra et al. 2001; Shanmugalakshmi et al 2003; Shankarkumar 2003; Kankonkar et al. 2003).

One of the characteristic properties of HLA diversity in human population is the phenomenon of linkage disequilibrium, the non-random association of particular alleles at HLA loci. Certain haplotypes are very much more frequent than any other combination of alleles (Shankarkumar et al. 2003). Strong linkage disequilibrium between closely linked loci may be due to lack of cross - over between the loci and more likely selection for a particular combination of allele (Apple and Erlich 1996). In principle population admixture may also create linkage disequilibrium patterns but that is unlikely to account for extensive disequilibrium observed in human populations. Thus elucidation of the extended haplotypes in the Nadar population by molecular typing for newly identified antigens will reveal the HLA allelic diversity and enable to identify newly arisen favorable variant alleles co-existing in the population. Further it will help in identifying the allelic mismatches in the allogenic Bone marrow transplantation unrelated donors.

REFERENCES

- Apple RJ, Erlich HA 1996. HLA class II genes: Structure and diversity. In: MJ Browning, AJ McMichael (Eds.): *HLA and MHC: Genes, Molecules and Function*. Oxford: BIOS Scientific Publishers. Pp. 97 – 112.
- Balakrishnan K, Pitchappan RM, Suzuki K, Shankarkumar U, Santhakumari R, Tokunaga K 1996. HLA affinities of Iyers, a Brahmin population of Tamil Nadu. South India. *Hum Biol*, **68**: 523-537.
- Bhasin MK, Walter H, Danker-Hopfe H 1994. *People of India: An Investigation of Biological Variability in Ecological Ethno-Economic and Linguistic Groups*. Delhi: Kamla-Raj Enterprises.
- Bhasin MK, Walter H 2001. *Genetics of Castes and Tribes of India*. Delhi: Kamla-Raj Enterprises.
- Boyum A. 1968. Separation of leucocytes from blood

- and bone-marrow. *Scan J Clini Lab Invest*, **21(Suppl)**: 97.
- Brahmajothi V, Pitchappan RM, Kakkanaiah VN et al. 1991. HLA association of pulmonary tuberculosis in South India. *Tubercle*, **72**: 123-132.
- Hardgrave R 1969. *The Nadars of Tamil Nad, The Political Culture of a Community in Change*. Berkeley: University of California Press.
- Haimendorf C.von.Furer 1948. Culture strata in Deccan. *Man*, **48**: 87 – 90.
- Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T 1992. Allele and Haplotype frequencies for HLA and Complement Loci in Various Ethnic Groups. In: K Tsuji, M Aizawa, T Sasazuki (Eds.): *HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference*. Vol. 1. Oxford: Oxford University Press. Pp 1066-1220.
- Kankonkar S, Jeyanthi G, Singhal BS, Shankarkumar U 2003. Evidence for novel DRB1*15 allele associations among clinically definite multiple sclerosis patients from Mumbai. *Hum Immunol*, **64**: 478-482.
- Kaufmann J 1996. Evolution of Major Histocompatibility Complex and MHC like molecules. In: MJ Browning, AJ McMichael (Eds.): *HLA and MHC: Genes, Molecules and Function*. Oxford: BIOS Scientific Publishers. Pp 1-17.
- Malhotra KC 1978. Morphological composition of the people of India. *J Hum Ecol*, **7**: 45-53.
- Manikasundari M, Sevaraj P, Pitchappan RM 1984. Studies on T cells of the lizard *Calotes versicolor*: adherent and non-adherent populations of the spleen. *Dev Comp Immunol*, **8**: 367-374.
- Mehra NK, Jaini R, Balamurugan A, Kaur G 2001. HLA A*02 diversity in North Indians: Predominance of HLA A*0211. *Tissue Antigens*, **57**: 502-507.
- Menon TM 1996. *Encyclopedia of Dravidian Tribes*. Thiruvananthapuram: International School of Dravidian linguistics.
- Papiha SS 1996. Genetic variation in India. *Hum Biol*, **68**: 607-628.
- Pitchappan RM 2002. Castes, migration, immunogenetics, infectious diseases and South India. *Community Genetics*, **5**: 157-161.
- Pitchappan RM, Kakkanaiah VN, Rajasekar R, Arulraj N, Muthukaruppan VR 1984. HLA antigens in South India: I Major groups of Tamil Nadu. *Tissue Antigens*, **24**: 190-196.
- Rajasekar R, Kakkanaiah VN, Pitchappan RM 1987. HLA antigens in South India II: selected caste groups of Tamil Nadu. *Tissue Antigens*, **30**: 113-118.
- Ravikumar M, Dheenadhayalan V, Rajaram K et al. 1999. Associations of HLA DRB1, DQB1 and DPB1 alleles with pulmonary Tuberculosis in South India. *Tuber Lung Dis*, **79**: 309-317.
- Risley H 1969 [1915]. *The People of India*. Delhi: Orient Book Reprint Corporation.
- Shanmugalakshmi S, Balakrishnan K, Manoharan K, Pitchappan RM. 2003 HLA DRB1*, DQB1*in piranmali kallars and Yadhavas two Dravidian speaking castes of Tamil Nadu South India. *Tissue Antigens*, **61**: 451-464.
- Shankarkumar U, Gupte SC, Gupte SS, Pednekar SV, ghosh K, Mohanty D 1998. Frequency and potential application of HLA antibodies from pregnant women from Mumbai. *J. Bio Sci*, **23**: 601-604.
- Shankarkumar U, Ghosh K, Mohanty D 2001. HLA antigen distribution in Maratha community from Mumbai, Maharashtra, India. *Int J Hum Genet*, **1**: 173-177.
- Shankarkumar U, Ghosh K, Mohanty D 2002a. HLA B27 diversity from Western India. *Tissue Antigens*, **60**: 98-101.
- Shankarkumar U, Ghosh K, Mohanty D 2002b. Defining the allelic variants of HLA A19 in the western Indian population. *Hum Immunol*, **63**: 779-782.
- Shankarkumar U 2003. HLA A*02 and B associated haplotype diversity in Indians. *Br J Biomed Sci*, **60**: 109-112.
- Shankarkumar U, Devaraj JP, Ghosh K, Mohanty D 2003. HLA A*02 allele frequencies and B haplotype associations in western India. *Hum Immunol*, **64**: 562-566.
- Trachtenberg EA, Erlich HA, Rickards O, Destefano GF, Klitz W 1995. HLA class II Linkage disequilibrium and haplotype evolution in cayapa Indians of Ecuador. *Am J Hum Genet*, **57**: 415-424.
- Terasaki PI, McClelland JD 1964. Microdroplet assay of human serum cytotoxins. *Nature*, **204**: 998-1000.