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Study of Selected HLA-A and -B Antigens by PCR-SSP Method in Bengali Population of Siliguri and Adjoining Areas of West Bengal

Bisu Singh, Ganesh Chandra Mallick, Sikta Bandopadhyay, Chitta R. Nayak and Tapas Kumar Chaudhuri*

Cellular Immunology Laboratory, Department of Zoology, University of NorthBengal, Siliguri 734 013, West Bengal, India
E-mail: zoology_nbu@rediffmail.com

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ABSTRACT Human leukocyte antigen is a highly polymorphic gene cluster which has made it a valuable tool in the population genetic studies. In this study one hundred individuals belonging to Bengali community of Siliguri sub-division of West Bengal were studied for 20 of the HLA-A and B loci. The HLA alleles were analyzed by using sequence specific primers for polymerase chain reaction (PCR-SSP). The result showed the increase frequency of HLA-A*02, -A*11, -A*24, -A*31, - B*07, -B*08, and -B*37 amongst the tested alleles. The notable observation of this study is the higher incidence of HLA-B*37 and -B*08 which is observed to be the highest amongst the Indian populations. The two-locus haplotype analysis revealed significant positive linkage disequilibrium for A*01-B*37, A*01-B*40, A*29-B*40, A*30-B*51, A*31-B*40. The study provides the HLA data of the Bengali population of this region.

INTRODUCTION

West Bengal is on the eastern bottleneck of India, stretching from the Himalayas in the north to the Bay of Bengal in the south and occupies only 2.7% of the India's land area, and supports over 7.8% of Indian population (West Bengal Human Development Report 2004). West Bengal lies between 85° 50' and 89° 50' east longitude, and 21° 10' and 27° 38' north latitude. The majority of the population of the state consists of Bengalis (http://www.infobengal.com).

Bengali race is a mixed breed of population broadly of Dravidians, Mongols and Aryans. There is some amount of admixture of aboriginals like Mundari and Santhals (Mazumdar 1998).Thus it may be stated that Bengali as a community is not too homogeneous (Raha 1975).

Siliguri is a cosmopolitan city of the sub himalayan region of West Bengal, consisting of Marwari, Punjabi, Bihari, Gorkha and Bengali communities, with Bengalis being the most prominent community here. Siliguri has seen waves of massive immigration over the years. The present Bengali population consists mostly of immigrants from Bangladesh and Assam (Kumar 2006).

Due to its high polymorphism, tight linkage among the loci and non-random association of alleles, Human leukocyte antigen (HLA) has become interesting from the perspective of population genetics. All the regions of HLA are known to be highly polymorphic, consisting of large number of closely linked genes that can be further split into many allelic types. Therefore the importance of this system in the study of polymorphism and their significance in population selection and survival and in providing clues to mechanism of generation as well as maintenance of this variability within the populations is immense (Srivastava 2007a).

HLA profiles of various populations are available from various parts of India (Mehra et al. 1986; Selvakumar et al. 1988; Balakrishanan et al. 1996; Agarwal et al.1999; Chhaya and Shankarkumar 2001). The HLA data are available for some of the ethnic and tribal populations of West Bengal (Debnath et al. 2006a; Debnath et al. 2006b; Srivastava 2007b; Agrawal 2008). To our knowledge only one study has been carried

*Corresponding author:
Prof. T.K.Chaudhuri
Cellular Immunology Laboratory, Deptt. of Zoology
University of North Bengal, Rajarammohanpur
Siliguri -734013, West Bengal, India
Telephone: +91-353-2699124(O), 9434377127(M)
Fax: +91-353 2699001
e-mail: dr_tkc_nbu@rediffmail.com

out to study the HLA profile in Bengalis by Raha (1975).This study has been carried out long before the advent of the modern molecular typing methods. On the other hand from our literature review it is obvious that HLA study has not been carried out in the Bengali population of Siliguri. Moreover, very recently Ali et al. (2008) have reported the HLA allele frequencies in Bangladeshi Bengalis. Therefore, the present study has been carried out to study the HLA Class I profile of Indian Bengali population residing in Siliguri, West Bengal, with the help of modern PCR based molecular typing technique.

MATERIALS AND METHOD

Samples: Blood samples were collected from 100 unrelated healthy Bengalis residing in and around Siliguri sub-division of West Bengal. Three-generation pedigree charts were prepared to assure that the samples were unrelated. All the participants provided their written consent for giving the blood samples after the study procedure were explained. The study has been carried out according to the principles of the Declaration of Helsinki (1964).

***HLA Molecular Typing*:** In this study 20 of the HLA alleles from HLA-A and -B were selected for the molecular typing in all individuals who were included in the study. DNA was extracted from peripheral mononuclear cells of the blood by the Phenol Chloroform method as described by Comey et al., 1994. Low-resolution molecular typing using polymerase chain reaction-based sequence-specific primer technique was performed for detecting HLA-Class I alleles. The typing and sequence information of primers were taken from Bunce et al. (1995) and 12th IHWC. The primers, Taq polymerase, nucleotide etc were obtained from Bangalore Genei, India. The sequence of the some of the primers which was used for genotyping HLA is as follows:- HLA-A*11; forward primer (5'ACGGAATGTGAA GGCCCAG3), and reverse primer (5'CTCTCTG CTGCTCCGCCG3'), HLA-B*08 forward primer (5'GACCGGAACACACAGATCTT3') and reverse primer (5'CCGCGCGCTCCAGCGTG3'). In general 25µl of reaction mixture in 1x PCR buffer, 200µM of each of dNTP, 1.5mM $MgCl_2$ 0.4µM of forward and reverse primers, 100ng of genomic DNA and 1unit of Taq polymerase. The amplifications were accomplished on a thermal cycler (Perkin Elmer, USA). PCR reactions are subjected to 30 cycles, each consisted of 94°C for 30sec., 60°C for 1min. and 72°C for 1min. with initial denaturation step of 2min. and final extension of 2min.

Statistical Analysis: Phenotype frequencies were calculated by direct count. Gene frequency was calculated by the formula $P=1-\sqrt{1-F}$, where F=frequency of allele. SPSS 15.0 software was used for calculating frequencies of different alleles. Haplotype frequencies were calculated by direct counting. The linkage disequilibrium was calculated from a 2x2 table and indicated as a delta value. The significance of this value was tested using the χ^2 test.

RESULTS

Allele frequencies of HLA-A and HLA-B loci of Bengali population studied are presented in table 1. The frequency of the alleles A*02, A*11, A*24, A*31, B*07, B*08, B*18 and B*37 were found to be highest among all the alleles tested.

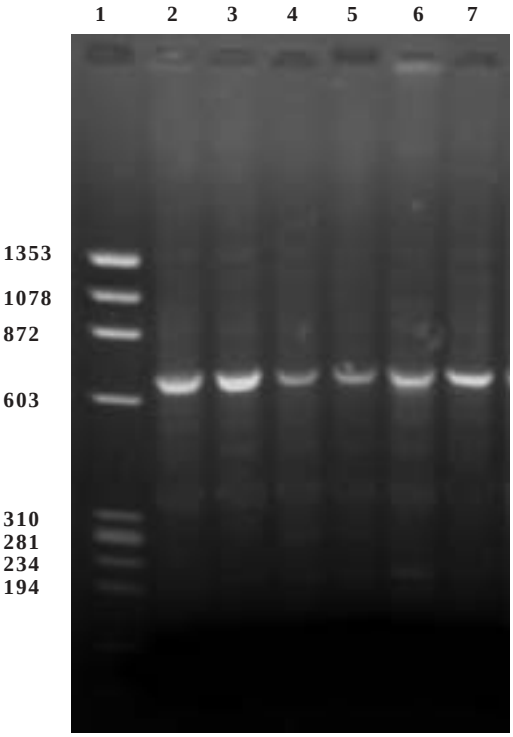

Fig. 1. Electrophoregram showing the result of HLA-B*07 (619 bp)
Lane 1, fX174 marker
Lane 2-7, amplified HLA gene

Among HLA-A group HLA-A*11 with frequency of 24% is found to be the most frequent allele followed by A*02 (20%), A*24(17.50%), A*31(18.50%) and among HLA-B alleles B*08 (22%),B*07 (17%) (Fig. 1), B*18 (14.50 %), B*37 (17 %) were found to have increased frequency.

Most common haplotype with significant positive linkage disequilibrium are shown in table 2. The two locus haplotype analysis revealed significant positive disequilibrium for A*01-B*37($p<0.01$), A*01-B*40, A*29-B*40, A*30-B*51, A*31-B*40($p<0.05$).

DISCUSSION

In the history of the Indian subcontinent, the most important invasion was by the West Asian semi nomadic tribes around 2000 BC. Some of these tribes migrated west and populated parts of Europe, whereas others migrated east towards Persia and over the Hindukush Mountains into the Indian subcontinent. Here they subjugated the dark skinned pre-Aryan (Dravidian) inhabitants of the Indus Valley civilization (Wolpert 2000). Since then, repeated invasions over the centuries by the Persian, Greeks, Turks, Arabs and Mongols have added to the genetic and cultural diversity of the Indo-Pak sub-continent and have a large amount of genetic admixture thus there may be certain level of shared ancestry (Mohyuddin et al. 2002).

In the present investigation frequency distribution of HLA-A and –B loci is studied among the Bengali population of Siliguri. Table 3 and 4 present the comparison of frequency distribution of HLA antigen among different populations. It has been observed that the frequency of A*02 and A*11 is uniformly high in all the populations like Sikh (Babita and Usha 2004) , Gujarathi, Maharastrian (Kankonkar et al. 2004) , different caste group of western India (Shankarkumar et al. 2001) and North India (Mehra et al.1997). Our previous study on Gurkha population also revealed the higher allele frequency of A*02 and A*11 (Debnath et al. 2006a). However, when the frequency profile of Bengalis were compared with other major world populations it was found that HLA-A*02 and A*11 were also higher in Greeks (Pachoula-Papasteriadis et al.1989).The higher frequency of A*24 observed in the present study is also observed in the majority of the South Indian (Thomas and Banerjee 2005), Northern Indian populations (Rajalingam et al. 2002) and Bangladeshi Bengali population (Ali et al. 2008). The higher frequency of A*31 is also observed in some of the South Indian populations (Vettriselvi et al. 2006).

Among the HLA-B alleles the higher frequencies of B*07 and B*08 is also observed in Sikhs (Babita and Usha 2004).In the North Indian population HLA-B*07 is the third highest frequency allele and is present at highest frequency in the Western European populations (Thomas and Banerjee 2005). HLA-B*37 is also found in Adiya, Ezhava of Kerala India (Thomas and Banerjee 2005) but its frequency is not as high as has been observed in the present investigation. The observed frequency of B*37 (17%) in this study is perhaps the highest among all the populations studied so far.

Table 1: Gene frequencies of HLA-A and-B antigens in Bengali population from Siliguri, West Bengal.

Allele	*Gene frequency*	*Gene frequency %*	*Standard error of gene frequency*
A*01	0.0808	8.0761	2.7247
A*02	0.1056	10.5573	3.0729
A*03	0.0228	2.2759	1.4913
A*11	0.1282	12.8220	3.3433
A*23	0.0356	3.5635	1.8538
A*24	0.0917	9.1705	2.8861
A*25	0.0408	4.0834	1.9791
A*26	0.0808	8.0761	2.7247
A*29	0.0540	5.3956	2.2593
A*30	0.0673	6.7262	2.5048
A*31	0.0972	9.7227	2.9627
B*07	0.0890	8.8957	2.8468
B*08	0.1197	11.9659	3.2456
B*18	0.0753	7.5338	2.6394
B*21	0.0151	1.5114	1.2201
B*37	0.0890	8.8957	2.8468
B*40	0.0540	5.3956	2.2593
B*42	0.0050	0.5013	0.7062
B*44	0.0025	0.2503	0.4997
B*51	0.0566	5.6602	2.3108

The frequent alleles such as A*02 and A*11 and A*24 observed in the present investigation were also found to be in higher incidence amongst the Bangladeshi Bengalis. Among HLA-B locus alleles the most common alleles of Bangladeshis such as B*44, B*40, B*51 were not found to be in higher frequencies amongst the Bengalis of the present study. Although the past history suggests the Bengalis of Bangladesh and West Bengal are closely related the differences seen here may be attributed to founder effect and genetic drift.

Table 2: Haplotypes showing significant significant linkage disequilibrium in Bengali population from Siliguri, West Bengal

Haplotype	*HF/1000*	*1000"x*	χ^2
A1-B37	499	96	6.861**
A1-B40	347	76	5.270*
A29-B40	366	78	4.237*
A30-B51	179	58	4.904*
A31-B40	17	82	6.614*

Note: *= p<0.05, **=p<0.01
HF=Haplotype frequency per 1000.
"=Delta

Table 2 presents the observed two locus hyplotype frequencies among Bengalis. The most frequent haplotype A1-B37, observed in the present investigation is also observed among Mumbai Marathas (Shankarkumar et al. 2001) and among the world population it is observed in Korean (Lee et al. 2005), Northern Ireland (Williams et al. 1999), North Western Islands (Spinola et al. 2002) and Azores (Spinola et al. 2005).

To our knowledge this study is the first of its kind to study the HLA status of the Bengali population of Siliguri, West Bengal using modern PCR based molecular typing method. The study presents preliminary HLA data which may be valuable to bone marrow transplantation registries and are useful in the study of molecular anthropology.

CONCLUSION

The distribution of HLA alleles among the Bengali population of Siliguri showed similarity with the other Indian populations as well as to Bangladeshi Bengalis. The most striking observation of this investigation is the high incidence of HLA-B*08 and B*37 which are observed to be highest when compared with other Indian populations.

RECOMMENDATION

Large amount of data involving both HLA Class I and Class II will be required to throw light to the phylogenetic history of Bengali population of this region.

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Table 3: Comparison of gene frequencies of HLA-A with the other populations

	Present study N=100	*Toto N=40*	*Gurkha N=50*	*Marathas N=289*	*Sikhs N=404*	*Bangladeshi Bengali N=141*
HLA						
A*01	0.0808	0.0253	0.01	0.1434	0.1212	0.0491
A*02	0.1056	0.2254	0.14	0.1569	0.142	0.0756
A*03	0.0228	0.0780	0.062	0.0430	0.1212	0.0179
A*11	0.1282	0.0917	0.1633	0.1227	0.1058	0.0813
A*23	0.0356	0.0646	0.01	0.0016		0.0036
A*24	0.0917	0.0382	0.02	0.0583		0.0852
A*25	0.0408	0.0513	0.0202			0.0197
A*26	0.0808	0.0780	0.04	0.0130		0.0161
A*29	0.0540	0.0382	0.000	0.0146		0.0071
A*30	0.0673	0.0513	0.01	0.0016		0.0036
A*31	0.0972	0.0382	0.0513	0.0081		0.0233

Table 4: Comparison of gene frequencies of HLA-B with the other populations

	Present study N=100	*Toto N=40*	*Gurkha N=50*	*Marathas N=289*	*Sikhs N=404*	*Bangladeshi Bengali N=141*
HLA						
B*07	0.0890	0.1197	0.041	0.1221	0.0626	0.0251
B*08	0.1197	0.0780	0.0304	0.0303	0.0706	0.0036
B*18	0.0753	0.0780	0.073	0.0117	0.0137	0.0071
B*21	0.0151			0.0218	0.0213	
B*37	0.0890	0.0126	0.041	0.0252	0.0175	0.0161
B*40	0.0540	0.0513		0.1033	0.0600	0.0341
B*42	0.0050					
B*44	0.0025	0.0646	0.073	0.0424		0.0472
B*51	0.0566	0.0646	0.0945	0.0235		0.0306

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