Annals of Pharma Research
ISSN: 2347- 1956
Research Article

COMPARATIVE ANALYSES OF THE ABO, KIR AND HLA LOCI AMONG THE RAJBANSHIS OF NORTH BENGAL REGION, INDIA.

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ABSTRACT

The sub-Himalayan Terai and Dooars region of North Bengal, India, is a hotspot of socio-cultural and ethnic diversity. One of the major caste populations present dominantly in this region is the Rajbanshi caste. Although a good numzber of opinions were forwarded regarding their origin, yet their genetic identity is still an unsolved question. In the present study, we have aimed to analyze the ABO blood group system in the Rajbanshis of Terai and Dooars region of North Bengal and also have compared our observations with that of the predictions from already published studies on KIR and HLA diversity of the Rajbanshis. The frequencies of the A, B and O alleles in the studied population were found to be 0.1751 0.2313 and 0.5937 respectively. From our study based on the ABO, KIR and HLA loci, we came to the conclusion that although the Rajbanshis have genetic background similar to the other Indian populations, yet the effect of mongoloid influence on their genetic structure can be well documented.

Keywords: Rajbanshi population, genetic diversity, HLA, KIR, Blood Group

INTRODUCTION

The present genetic structure of India has resulted from contributions by several waves of human migrations and gene flow [1] forming a hierarchial society stratified into tribes and caste populations. The sub-Himalayan Terai and Dooars regions of East India constitute an important geographic location due to their close proximity with the eastern territories of the Himalaya. Ethnically these regions are extremely diverse, inhabited by the populations belonging to different linguistic groups such as the Tibeto-Burman (TB), Austro-Asiatic (AA), Indo-European (IE) and Dravidian (DR). On one side of East India is the north-eastern part of the country which has been an important corridor of human dispersals while Nepal Himalayas on the other side acted as a barrier for bidirectional gene flow [2-3].

One of the population groups, comprising 18.5% of the total Scheduled Caste population of the state of West Bengal as per the 2001 census of Government of India is the Rajbanshi population. It comprises the major caste population of North Bengal region, a part of East India. In addition to this, a sizeable population of Rajbanshis also lives in the sub-Himalayan regions of Nepal as well as North-east India including Assam. The Rajbanshis represent one of the oldest and indigenous populations of Terai and Dooars regions with rich cultural heritage, unique linguistic and social background. Linguistically Rajbanshis belong to IE-speaking group; however, historic evidences indicate their tribal connection [4]. Thus the Rajbanshi population is of considerable interest for genetic studies as many questions arise with regard to its origin and genetic background.

Blood groups beside being a valuable tool in blood transfusion, forensics and paternity determination, is also one of the most simple and important genetic markers in studies of human population variations [5]. The ABO blood grouping system in human, discovered by Landsteiner in 1901 [6] and the Rh system defined by Landsteniner and Wiener in 1941 [7], together proved very useful for blood transfusion purposes. Although all the human populations share the same blood group systems, their frequencies differ markedly in different ethnicities across the world enhancing its importance in genetic research and in tracing anthropological and ancestral relations of human. Thus it makes a strong sense to evaluate the frequencies of ABO and Rh blood groups in different ethnic populations of our country including the Rajbanshis.

In the present study, we have investigated the ABO blood group frequencies in the Rajbanshi population and to understand the genetic relationships of Rajbanshis with other Indian populations, we have performed a comparative analysis based on genetic variations of Human Leukocyte Antigen (HLA), Killer cell Ig-like Receptors (KIR) and ABO blood group system in the Rajbanshis.

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MATERIALS AND METHODS

Blood samples were collected from 78 unrelated volunteers belonging to Rajbanshi caste population with prior informed consents from the donors. All the required information regarding each subjects were collected using a brief questionnaire. The Rajbanshi samples were screened from the Terai and Dooars region of Coochbehar ($26^{o}20'$N and $89^{o}29'$E) and Jalpaiguri ($26^{o}32'$N and $88^{o}46'$E) districts which are located in the northern region of the state of West Bengal. The blood samples were collected from the subjects under aseptic condition ABO and Rh blood groups testing were done by open slide method following the instruction of the manufacturer using anti-A, B, and D sera (Tulip Diagnostics, Goa, India). All samples were tested for the ABO blood groups. The allele frequencies for both the systems were calculated according to the method of Mourant *et al.,* (1976) [8].

The ABO blood group frequency data of populations to be compared with that of the Rajbanshi population were extracted from previously published reports as follows: Garo and Rabha [9], Oraon, Munda and Kharia [10], Manipur Brahmins and Meltei [11], Koch population of Assam [12], OBC population of Uttarpradesh [13], Rajasthani Nomadic Tribe [14], Vishwakarma population of Mysore [15] and Kunbis population [16]. The phenotypic frequencies of the ABO blood groups in the Rajbanshis were compared with that of the reference populations using Kyplot 2.0 beta 15 software. Principal Component Analysis was also computed based on the allele frequencies of the ABO blood grouping system in the Rajbanshis and other comparator populations using Minitab 16.0 statistical software. Hierarchial cluster analysis was also performed based on allele frequencies of the ABO system of the Rajbanshis and previously mentioned populations using SPSS version 15.0.

The KIR genotypic data of the Rajbanshi was extracted from Guha *et al.,* 2013 [17] and that of other comparator populations were obtained from: South Indian Paravar and Kanikar populations [18], North Indian [19], Maharashtrian and Mumbai parsis [20], Han Chinese [21], Japanese [22], British [23], Northern Irish [24], Afro–American [25], Afro-Caribbean [26], Chiriguanos and Wichis [27]. Based on the linkage disequilibrium values, two frequently occurring gene clusters were recognized within the KIR gene complex [28]. The centromeric or 'C4' cluster of the KIR gene complex consists of KIR2DS2-2DL2-2DS3-2DL5 genes and is located at the centromeric half, while the telomeric or 'T4' cluster located at the telomeric half of the KIR complex consists of KIR3DS1-2DL5-2DS1- 2DS5 genes. The Bx genotypes were further subdivided into four subsets based on the arrangement of the C4 and T4 clusters: C4Tx (C4 present but T4 absent), CxT4 (c4 absent but T4 present), C4T4 (both C4 and T4 present), and CxTx (both C4 and T4 absent). The frequencies of these four subsets in the Rajbanshi population and that of the other comparator population were compared using Kyplot 2.0 beta 15 software.

The HLA data of the Rajbanshis and other comparator populations (i.e. Kayastha, Rastogi, Vaish, Shia, Sunni, Lachungpa and Mech) were extracted from Agarwal *et al.,* 2008 [29]. Genetic Distances (Nei's D_A) were calculated in between the Rajbanshi and the comparator populations in order to explore the genetic relatedness of the Rajbanshis with the other populations.

RESULTS

The distributions of the ABO and Rh blood group systems in the Rajbanshi population of the Terai and Dooars regions of North Bengal are shown in Table 1. In the 78 samples analyzed from the Rajbanshi population, O blood type has the highest number of representatives (28) followed by B (25), A (18), and AB group (7) respectively (Table.1). The overall phenotypic frequencies of ABO blood groups were O>B>A>AB in the Rajbanshis (Fig. 1). The allelic frequencies of A, B and O alleles were 0.1751 0.2313 and 0.5937 respectively. The gene frequencies of Rh D and Rh d were found to be 0.886 and 0.114, respectively (Table 1). The comparison of the ABO blood group distribution in the Rajbanshis with that of other Indian populations has been shown in Figure 2. It can be clearly understood from Figure 2a that the Rajbanshis have the lowest phenotypic frequency of A blood type (23.06%, Table 2) compared to the other populations. Moreover the frequency of the AB phenotype (8.97%) was also low among the Rajbanshis just being greater than the Maratha Kunbis (8.33%) and Rajasthani Nomadic tribe (8.60%) respectively. From figure 2b it can be concluded that the 'O' allele frequency (r) tends to remain higher than the other blood group alleles (A and B) in the Indian populations. The Rajbanshis also have higher values of 'r' (0.594, Table 2) like that of other Indian populations and they have the second highest frequency value next to the Koches (0.629) in case of 'O' alleles.

Table.1: Distribution of the ABO and Rh blood group and their allele frequencies in the Rajbanshi populations.

Blood Group	Observed Number	Phenotypic Frequency	Allele frequencies
O	28	35.897	r[O]=0.594
A	18	23.077	P[A]=0.175
B	25	32.051	q[B]=0.231
AB	07	8.974	
Rh D +ve	77	98.717	0.886
Rh d	01	1.282	0.114

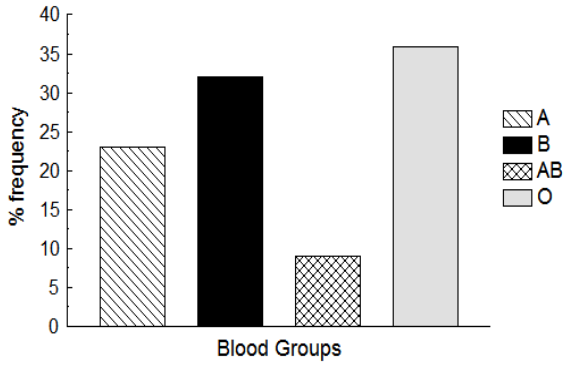

Figure.1: Phenotypic frequencies of the different ABO blood groups in the Rajbanshis.

Table.2: Comparison of the ABO blood group distribution in the Rajbanshis and other comparator populations of India.

Population	A	B	AB	O	p	q	r
Rajbanshi	23.08	32.05	8.97	35.90	0.175	0.231	0.594
Garo	29.86	32.64	11.81	25.69	0.236	0.255	0.507
Kunbis	27.02	33.06	8.33	31.04	0.198	0.235	0.567
Viswakarma	26.57	23.78	11.89	37.76	0.213	0.195	0.592
OBC	23.66	36.81	6.85	32.676	0.167	0.251	0.582
Rajasthan Nomads	25.5	40.4	8.6	25.6	0.209	0.286	0.505
koch	26.32	31.58	4.38	37.72	0.169	0.201	0.629
Manipur Brahmimns	35.1	18.54	11.59	34.77	0.267	0.162	0.571
Meltei	31.58	25.36	11.96	31.1	0.247	0.207	0.546
Oraon	28.9	36.5	12.5	22.1	0.242	0.293	0.467
Munda	32.3	35.4	10.4	21.9	0.265	0.285	0.450
Kharia	37	33.3	9.3	20.4	0.300	0.275	0.425
Rabha	32.23	30.16	12.26	25.34	0.255	0.241	0.503

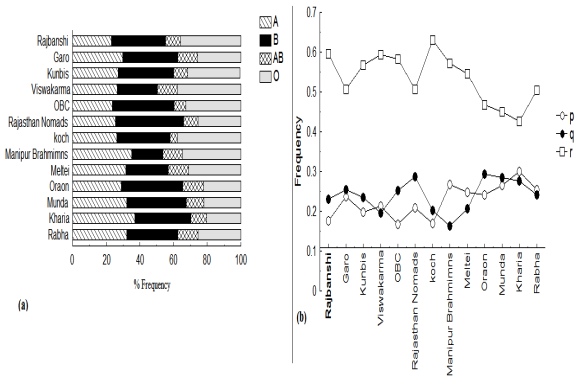

Figure.2: (a) Comparison of the blood group phenotypic frequencies of the Rajbanshis with that of other populations of India. (b) Comparison of the frequencies of the ABO blood group alleles between the Rajbanshis and other Indian populations.

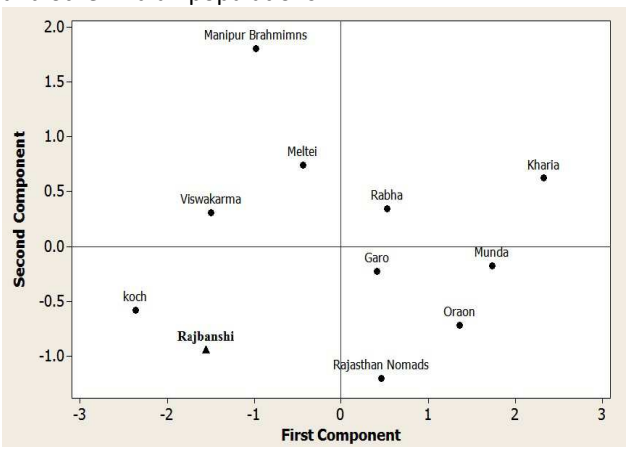

Figure.3: Principal Component Analyses (PCA) to compare the ABO blood group allele frequencies in the Rajbanshis with other comparator populations from India. The Rajbanshis has been marked with (▲) in the score plot of the PCA.

Principal Component Analysis (PCA) based on ABO allele frequencies was performed to compare the Rajbanshi populations with some of the previously reported populations from different parts of the country. The PC plot for the first two components has been shown in Figure.3. The first component accounted for 74.5% variability and the second accounted for 25.5% variability. It was observed in the score plot of the PCA that the Rajbanshis appeared in proximity to the Koches of Assam, North east India. In the plot the Rajbanshis shared the lower left quadrant with the Koches whereas other comparator populations are distributed in other quadrants throughout the graph. From the graph it was also evident that on one side the Rabhas and the Garos are in proximity while on the other the Oraons and the Mundas are closer to each other.

Table.3: Measures of Nei's genetic distances between the Rajbanshis and other neighbouring Indian populations.

	Rajbanshi	Garo	Rajasthani	Koch	Manipur	Meltei	Oraon	Munda	Kharia	Rabha
Rajbanshi	.000									
Garo	.109	.000								
Rajasthani	.110	.041	.000							
Koch	.047	.149	.156	.000						
Manipur	.117	.117	.152	.120	.000					
Meltei	.089	.063	.097	.114	.055	.000				
Oraon	.156	.055	.051	.200	.169	.117	.000			
Munda	.178	.071	.078	.220	.172	.125	.030	.000		
Kharia	.214	.106	.122	.253	.187	.149	.074	.044	.000	
Rabha	.121	.024	.064	.158	.105	.056	.065	.070	.096	.000

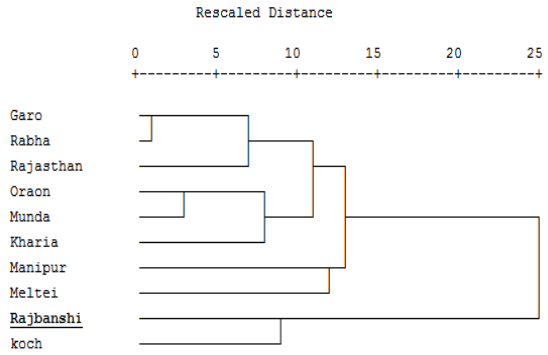

Figure.4: Hierarchial Cluster Analysis (HCA) of the Rajbanshis and other indian populations based on ABO allele frequencies

Hierarchial Cluster Analysis (HCA) was also performed based on the ABO allele frequencies and the resulting dendrogram (Figure 4) depicted similar result as that of PCA. The Rajbanshis formed a separate cluster with the Koches of Assam. The Oraon and the Mundas clustered with the Kharia while interestingly the Rajasthani nomadic tribe shared the same cluster with the Garos and the Rabhas. The Euclidean distances between the Rajbanshi and other comparator populations have been shown in Table 3 where it is clear that the Rajbanshis have the least distance with the Koches (0.047) followed by the Meltei group from Manipur (0.089). Interestingly the Rabha population which is generally considered to be genetically related to the Koches has greater distances from both the Koches and the Rajbanshis respectively.

KIR analyses:

All the four subsets of the Bx genotypes (CxT4, C4Tx, C4T4, and CxTx) were present in the Rajbanshi population (Table 4). The Rajbanshis have the lowest frequency of the Bx genotypes with C4Tx configuration (14.7%) compared to any other Asian Indian population. In contrast the Rajbanshis were also second lowest to the North Indians in the frequency of the C4T4 configuration of the Bx genotypes among the Indian populations. In the case of the CxTx subset, the North Indians have the highest representation (41.9%) among the Indian populations followed by the Rajbanshis (36%). Overall it was seen that the Rajbanshis along with the other Asian Indian populations have all the four subsets of the Bx KIR genotypes, thereby presenting a balancing condition in the Asian Indians (Figure 5). In total the Bx genotypic configuration of the Rajbanshis was more proximal to the Asian Indians compared to any other population clusters. Moreover the Rajbanshis have higher frequencies of Bx genotypes (87%) compared to AA genotypes (13%) which is also a characteristic feature of the Indian populations [17].

Table.4: Distribution of the four gene clusters of the Bx subsets in the Rajbanshis and other neighbouring Indian populations.

Population	Bx subsets			
	C4Tx	C4T4	CxT4	CxTx
Rajbanshi	14.7	10.7	18.7	36
Paravar	19.5	28.6	31.2	16.9
Kanikar	20	34.2	25.7	16.8
North Indian	23.7	8.4	21	41.9
Maharashtrian	22.8	16.1	16.4	21
Parsis	17.8	29.5	16.5	11.2
Chinese Han	1.2	2.5	23.6	17.5
Japanese	2.9	1	21	16.2
British	13.9	4.4	19.9	31.7
Northern Irish	16.3	8.3	21.2	18.1
Afro-American	20.7	3.4	6.9	39.6
Afro-Caribbean	19.6	0	9.9	34.8
Chiriguanos	0	1.9	50.3	16.9
Wichis	0	1	51.6	17.1

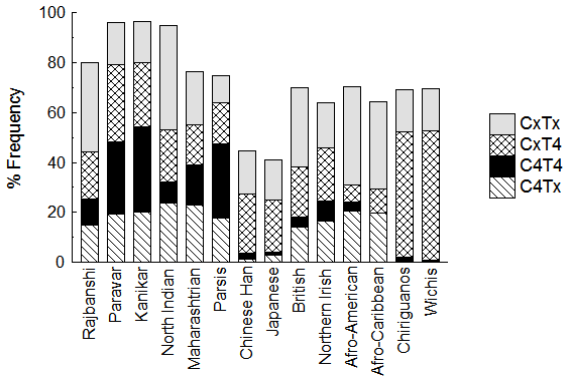

Figure.5: Comparison of the frequencies of the subsets of the Bx genotypes in the Rajbanshis with the other Indian populations.

HLA Analyses:

The frequencies of the HLA class II alleles in the Rajbanshis were shown in Table 5, from the table it was evident that in Rajbanshis within the DRB1 locus, DRB1*0701 have the highest frequency (13.90%) followed by DRB1*1501 (13.70%) and DRB1*1201 (10.20%) respectively. From the DQA1 locus, DQA1*0201 (26.50%) was the most frequent followed by the DQA1*0501 (14.20%) and DQA1*0102 (13.30%) respectively. Similarly within the DQB1 locus DQB1*0501 (29.08%) and DQB1*0301 (23.40%) are the most frequent alleles in the Rajbanshis. The genetic distance of the Rajbanshis (Table 6) when compared to other populations have shown that they have the least distance with that of the Mech (0.048) followed by the Lachung (0.068) which are the two tribals of the Tibeto Burman group, thereby showing the Tibeto-Burman connection of the Rajbanshis.

Table.5: Distribution of the HLA class-II allele frequencies in Rajbanshis.

Allele	% freq	Allele	% freq	Allele	% freq
DRB1*0101	3.06	DQA1*0101	6.12	DQB1*0201	16.30
DRB1*0301	5.60	DQA1*0102	13.30	DQB1*0301	23.40
DRB1*0401	3.06	DQA1*0103	8.16	DQB1*0302	1.50
DRB1*0402	0.50	DQA1*0104	7.70	DQB1*0303	2.04
DRB1*0701	13.90	DQA1*0201	26.50	DQB1*0401	1.03
DRB1*080X	9.20	DQA1*0301	12.20	DQB1*0402	1.03
DRB1*090X	3.06	DQA1*0302	1.53	DQB1*0501	29.08
DRB1*1001	5.70	DQA1*0401	1.53	DQB1*0502	2.55
DRB1*1101	9.70	DQA1*0501	14.20	DQB1*0503	1.50
DRB1*1103	1.03	DQA1*0601	8.70	DQB1*0601	19.00
DRB1*1201	10.20			DQB1*0602	2.55
DRB1*1202	1.00				
DRB1*1301	2.04				
DRB1*1302	3.60				
DRB1*1401	7.14				
DRB1*1404	2.04				
DRB1*1405	2.04				
DRB1*1501	13.70				
DRB1*1502	3.60				

Table.6: Nei's Genetic Distances in the Rajbanshis compared to other Indian populations based on HLA allele frequencies.

	Kayastha	Rastogi	Vaish	Shia	Sunni	Lachung	Mech	Rajbanshi
Kayastha	0							
Rastogi	0.085	0						
Vaish	0.051	0.017	0					
Shia	0.148	0.119	0.118	0				
Sunni	0.096	0.050	0.068	0.049	0			
Lachung	0.131	0.144	0.153	0.212	0.158	0		
Mech	0.128	0.144	0.157	0.199	0.160	0.029	0	
Rajbanshi	0.128	0.096	0.132	0.169	0.109	0.068	0.048	0

DISCUSSION

Many opinions have been expressed regarding the origin and genetic background of the Rajbanshis. Historically the Rajbanshis are considered to be the descendants of the Koches who are mongoloid in their origin. Sir H. H. Risley commented that the Rajbanshis are of Dravidian origin with considerable mongoloid admixture [30]. From both the PCA and the HCA analyses, it was evident that the Rajbanshis have a tendency to cluster with that of the Koches of Assam. The Koches significantly differed from the Rabhas in their blood group distribution [12]. Similar observation was also made by Das *et al.,* 1962 [31] who opined that the Rajbanshis (Koch) of Assam were more similar to lower caste group of Assam, than the tribes like the Garo, Kachari and the Rabhas. Our study also simulated the similar result and grouped the Rajbanshis with the Koches but have significant differences in their ABO allele frequencies from the Garos and the Rabhas. Thus the ABO blood group system in the Rajbanshis suggests their mongoloid connection. From the KIR genotypic analyses it was evident that the Rajbanshis have the KIR distribution similar to the Asian Indian populations and have a balancing condition of all the clusters of the Bx genotypes. Moreover based on gene frequencies of all the KIR genes the Rajbanshis have a tendency to huddle with the Asian Indian cluster [17]. However it has also been mentioned by Guha *et al.,* 2013 [17] that the Rajbanshis of North Bengal region have specific KIR genotypes that were found in the Tibetan population and has not been reported in any Indian population group. Thus it suggests that the Rajbanshis of the Terai and Dooars region of North Bengal have Indian specific genetic structure with mongoloid influence. On comparing the HLA frequencies in the Rajbanshis with that of other populations it was seen that the Rajbanshis have the least distance from two tribal populations of Tibeto-Burman inheritance i.e. the Mech and the Lachung. However the HLA allele frequencies in the Rajbanshis suggest that although influenced by the mongoloid element, their genetic background experienced extensive admixture [29]. Our view is also supported by the Y haplogroup diversity of the Rajbanshis [32] which suggests that the O3 haplogroup which is absent in Indo-European speaking caste populations from Eastern India is considerably present in the Rajbanshis and other Tibeto-Burman groups. Moreover, this O3 haplogroup in the Tibeto Burman populations dated older than the arrival of the Indo-European language thereby suggesting earlier Tibeto-Burman influence on the Rajbanshis.

Based on all these evidences from a number of genetic markers, our study suggests that although the Indo-European speaking Rajbanshis from Terai and Dooars region of North Bengal have Indian origin, the Tibeto-

Burman influence on their genetic background cannot be ignored. However this population has extensive genetic diversity and considerable admixtures and therefore further studies based on other available genetic markers [33] are required for detailed exploration of the genetic wealth of the Rajbanshis.

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