ALLELIC AND PHENOTYPIC DIVERSITY OF ABO AND RHESUS (D) BLOOD GROUPS IN THE MEDICAL AND DENTAL APPRENTICES OF A UNIVERSITY OF HARYANA, INDIA

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ABSTRACT

*Human blood group phenotypes are important for blood transfusion programs, disease association and population genetics studies. This study is designed to report the phenotypic and allelic distribution of ABO and Rhesus (Rh) groups in various caste groups of the students enrolled in Medical and Dental Colleges of SGT University. ABO and Rhesus blood groups and self-identified ethnicity was obtained from 570 medical students of this University. Ethnicity has been classified into six major (caste) groups: Jat, Yadav, Brahmin, Baniya, Punjabi and Others. ABO allelic frequencies were determined using the Bernstein method. Differences in phenotypic distribution of blood groups were assessed using the chi-square test. The frequencies of the antigens of blood groups ABO*O, ABO*A, ABO*B and ABO*AB in the target group observed were 31.4%, 24.04%, 38.25% and 6.32%, respectively; and Rhesus-positive was 92.63%. The allelic frequencies of ABO*O, ABO*A and ABO*B genes were 0.5752, 0.2578 and 0.16699 respectively. Phenotypic frequencies of the blood groups in the general study population and in the different ethno-linguistic groups were in agreement with Hardy–Weinberg equilibrium expectations (*$P > 0.05$*). This study provides the first data, based on ethnic distribution of ABO and Rhesus blood groups in the Medical students of Haryana and suggests that its general profile is similar to those of several sub- populations of the region. However, we found some significant differences in phenotypic distribution amongst major caste groups of Haryana; a close proximity was observed between Brahmin students of the present study with that of previously studied groups. Yadav students were found distanced from the other caste groups in the study. This data will contribute for blood donor recruitment policy and blood transfusion services in the Medical Colleges.*

KEYWORDS: Allelic diversity, ABO blood group, Rhesus factor,

INTRODUCTION

Blood, being a specialized connective tissue with complete and unchangeable identity, is useful in identity testing. ABO along with Rh antigens is the major clinically significant blood group antigens out of identified 400 blood grouping antigens for transfusion purposes and anthropological studies (Adeyemo*et al*., 2006). The credit for putting forward the ABO blood group system goes to Karl Landsteiner (Landsteiner&Weiner, 1940). The genes coding for ABO and Rh (D) are located on Chromosome nine and one respectively. The antibodies against red blood cell antigens are called agglutinins and individuals are divided into four major blood groups i.e. A, B AB and O, according the presence of these antigens and agglutinins (Ganong*et al*., 2005). The ABO system derives its importance from the fact that A and B are strongly antigenic; and anti A and anti B occur naturally in the serum of persons lacking the corresponding antigen(Bauer,1982). In addition, these antibodies are capable of producing haemolysis in vivo(Plapp& Beck, 1984). Since last four decades, these antigens are playing an important role in the studies related to evolution, diseases management and environment, blood transfusion, organ transplantation, forensic pathology, anthropology and establishment of ancestral relationship of human (Mollison*et al*.,1993) and also facilitates prevention of complications due to Rhesus Incompatibility (Olubayode*et al*., 2013).

Large biological data of Indian populations has been generated by anthropological studies, andis being extensively used to comprehend the population structure of India; however, few studies for the state of Haryana's population are available (Khurana 1956; Kushwaha*et al*.1990a, b; Yadav *et al*. 2001, Singh *et al*., 2013, Jaggi& Yadav, 2014). The earlier studies divulges that 72% Indian blood group belongs to the Indo-Aryan, 25% tothe Dravidian while only 3% belongs to theMongoloid and other ethnic groups (http://www.bloodbook.com/world-abo.html).The biological variations observed at serological and genetic level facilitates in establishing relationships amongst individuals and population groups. The uniqueness of Indian population structure lies in division of ethnic population groups on the basis of caste system.

Haryana (Sanskrit "the Abode of God"), is aNorth West landlockedstate of India. According to census 2011, Haryana's population is 2.53 billion, and comprises of 1.35 billion males and 1.18 billion females. Hindus for the major caste in Haryana and are

approximately88.23% of the population, they are followed by the Sikh 6% and the Muslims 5.78% (mainly Meos). Even though the sex ratio in India has undergone a commendable improvement since the past decade; Haryana, still has one of the lowest sex ratio (877 female to 1000 of males) when compared to the national sex ratio of 943:1000. The state'sdecreasing female sex population is a cause of concern for the state administration and NGOs working in this field.Haryana has a literacy rate of 75.55% with significant difference in literacy ratio of male and female (84.06: 56.91). Compounding this problem is Gurgaon, where SGT University is located, has the lowest sex (854/1000) ratio, amongst the districts, and has the highest literacy rate in state of 84.7%.

The present study was carried out with the aim of providing information on the distribution of ABO and Rhesus blood groups among Medical students in Shree Guru Gobind Singh Tricentenary (SGT) University, Gurgaon, Haryana, India and also to determine the most common blood groups for purpose of emergency blood transfusion.

MATERIAL AND METHODS

The study was conducted on the randomly selected medical and dental students (age group 18-22 years) of the SGT University, Gurgaon, Haryana, India. The blood samples were collected from 570 students after obtaining their consent. The samples were analysed for blood groups ABO and Rh(D). The basic techniques used were given by Bhasin and Chahal (1995) and the blood analysis was done using Eryclone monoclonal antibodies. The data were statistically analysed(expected frequencies and Chi square test forHardy Weinberg equilibrium (HWE) using S2ABO Frequency Estimator (http://alf1.cii.fc.ul.pt/~pedro/Soft/ABOestimator/). After analysis, students of the state were further divided into sub-categories as majority of them belong to prominent caste groups. The sub-categories in which these students were divided are: Jat, Yadav, Baniya, Brahmin, Punjabi and Other. Other group included students of other castes of Haryana and those hailing from other states. The students of other state group being statistically in-significant were excluded from the study. The evolutionary relationship among the studied populations was also calculated by DISPAN software (http://oat.bio.indiana.edu) and phylogenetic tree was constructed with Mega 6 software (Tamura *et al*. 2013). The major focus was concentrated to the genetic variation of the population.The dataset obtained were compared

with the other Indian populations already published in the literature to get the clear picture of the genetic makeup of the population and serological diversity of the population.

RESULTS AND DISCUSSION:

Blood samples of 570 medical students were analysed in this study. Amongst these students, there were 222 male and 348 female students. The scope of the study was restricted to the students of Haryana state only; and comprised of 179 males and 303 female students. It is interesting to note here; beside low sex ratio and literacy rate in females of Haryana, the number of fairer sex studying medical science was pretty high, as compared to the male gender.

The results of ABO blood groups of students including observed number, reflecting observed and expected frequencies is given in Table 1. Overall phenotypic frequency for the complete students was in the following order i.e. ABO*B> ABO*O> ABO*A> ABO*AB. The phenotypic frequencies were highest for ABO*B (38.6%) followed by ABO*O (30.29%)(Figure 1). The frequencies of ABO*A was observed as 25%, whereas frequency of ABO*AB group was observed to be comparatively very low (6.02%). Similar pattern was observed for Jat, Punjabi and Baniya students. However, variation was observed in Yadav and Brahmin students where the frequency of ABO*A blood group was higher than ABO*O.Similar observations of high frequency of ABO*A in Brahmin students in accordance of previous studies(Meitei *et al*., 2010, Yadav *et al*., 2011). The observed and expected allelic frequencies of ABO markers, their standard errors and the percentage of observed blood groups are also listed in Table 1. There was no significant variation between the observed and expected frequencies. The allelic frequencies observed for medical students belonging to Haryana were in the order of ABO*O (0.569148), ABO*B (0.258767) and ABO*A (0.172085). For Jat students, considerable variations were observed between observed and expected frequencies; however, no such variation was observed in the case of other students of Haryana.

Table 1. ABO Data for Medical and Dental Students of SGT University

Pop	Blood group	Observed numbers	Percentage frequency	Expected numbers	Allelic frequency	Standard errors
All Students	A	137	24.04	125.39	0.166988	0.011568
	B	218	38.25	206.94	0.257817	0.014000
	O	179	31.40	188.58	0.575195	0.015987

	AB	36	6.32	49.08		
	Total	570	100	569.99	1	
Haryana Students	A	121	25.01%	108.69	0.172085	0.012751
	B	186	38.59	174.25	0.258767	0.015246
	O	146	30.29	166.80	0.569148	0.017445
	AB	29	6.02	42.93		
	Total	482				
Jat Students	A	51	24.52	44.26	0.158750	0.018718
	B	81	38.94	74.59	0.250389	0.022895
	O	67	32.21	72.62	0.590861	0.026200
	AB	9	4.33	16.54		
	Total	208	100	208.01	1	
Yadav Students	A	42	28.97	39.40	0.218562	0.025828
	B	53	36.55	50.47	0.269037	0.028222
	O	36	24.83	38.07	0.512402	0.032687
	AB	14	9.66	17.05		
	Total	145	100.01	128	0.9999	
Brahmin Students	A	16	28.07	12.09	0.1677	0.0366
	B	26	45.62	22.33	0.2837	0.0460
	O	14	24.56	17.15	0.5486	0.0514
	AB	1	01.75	5.42		
	Total	57	100	56.99	1	
Punjabi Students	A	10	18.52	10.16	0.139159	0.034589
	B	20	37.04	20.15	0.254318	0.045245
	O	20	37.04	19.87	0.606524	0.050962
	AB	4	07.41	3.82		
	Total	54	100.01	54	1	
Baniya Students	A	2	18.52	2.31	0.086584	0.047954
	B	6	37.04	6.29	0.216899	0.073232
	O	9	37.04	8.73	0.696517	0.081401
	AB	1	07.41	0.68		
	Total	18	100.01	18	1	

Overall Heterozygosity(Table 2) observed was 0.577 for all the medical students, whereas, observed heterozygosity for Haryana students only, in these courses was found to be 0.58066. Highest heterozygosity was observed in Yadav Students 0.62216, followed by Brahmin students (0.60097). The least heterozygosity was shown in students of the Baniya community (0.4874).

Table 2.AvarageHetrozygosity of Various Caste Groups

Sr. No.	Caste Group	Average Heterozygosity	Sr. No.	Caste Group	Average Heterozygosity

1	All Medical Students*	0.577002	18	HaryanaBanjara	0.666621
2	Haryana students*	0.58066	19	HaryanaKumhar	0.64968
3	Jat students*	0.565758	20	HaryanaSaini	0.646378
4	Yadav Students*	0.62216	21	North Indian Saraswats	0.613036
5	Brahmin Students*	0.600972	22	Assam	0.557573
6	Punjabi Students*	0.558454	23	Andhra Pradesh	0.482271
7	Baniya Students*	0.487422	24	Goa	0.567205
8	Haryana Meos	0.58621	25	HP	0.628846
9	Haryana Sunni Muslims	0.600312	26	Jammu and kashmir	0.558857
10	HaryanaJat	0.577001	27	Kerala	0.599202
11	HaryanaAhir	0.582915	28	Maharashtra	0.522222
12	HaryanaRor	0.634935	29	MP	0.587732
13	Saini	0.602925	30	Orissa	0.531328
14	Kamboj	0.590859	31	Rajasthan	0.578474
15	Kumhar	0.564896	32	Sikkim	0.584421
16	HaryanaChamar	0.59656	33	UP	0.607711
17	HaryanaMullah	0.509395	34	West Bengal	0.570949
			35	Punjab	0.658973

- Data from current study

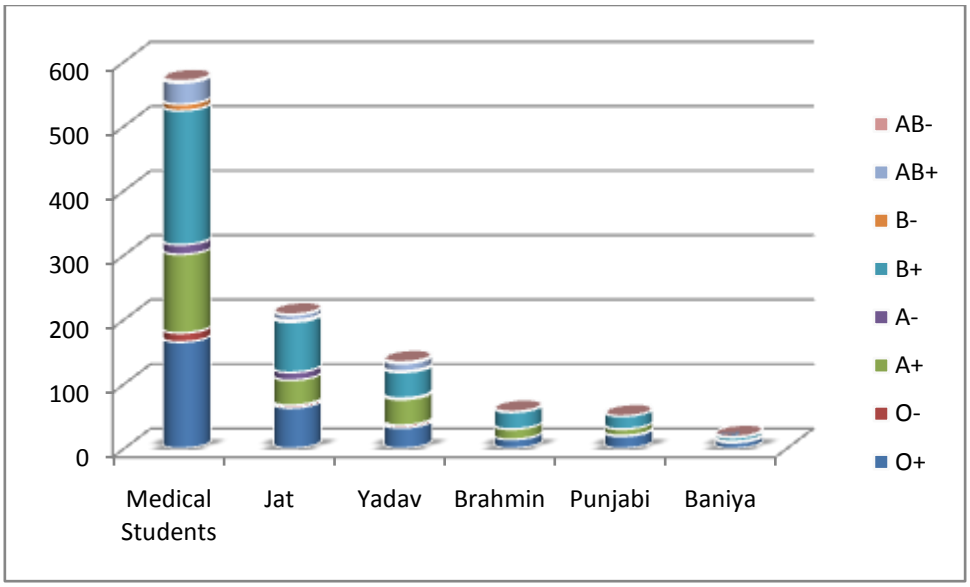

Figure 1. Community Based Blood Group Frequencies in Medical Graduates

Intra population comparison of populations under observation indicated the close proximity between Baniya and Punjabi students, whereas Jat, Brahmin and Yadav students were clustered on the other branch (Figure 2).

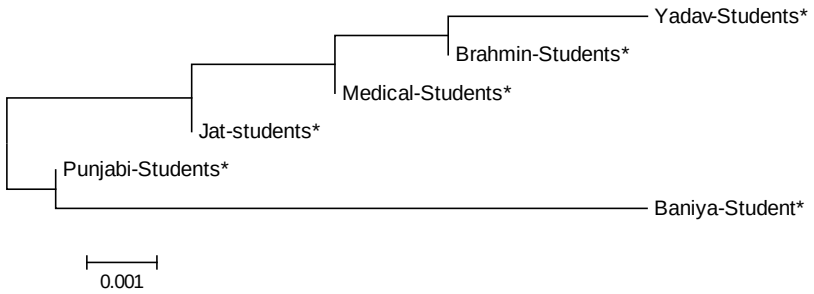

Figure 2.Evolutionary Relationships among the Studied Groups.

In comparative analysis of current data with thirteen earlier studies of Haryana populations (Figure 3), it was observed that Haryana students showed maximum genetic distance from Haryana MullahPopulation (Jaggi& Singh, 2014),but theleast genetic distance from Meos and Sunni Muslims(Singh *et al*., 2013). Baniya population had shown the maximum distance from the Banjara population (Jaggi& Yadav, 2014) of Haryana. The gene diversity (G_{ST}) per loci amongst the 19 populations was 0.0176.

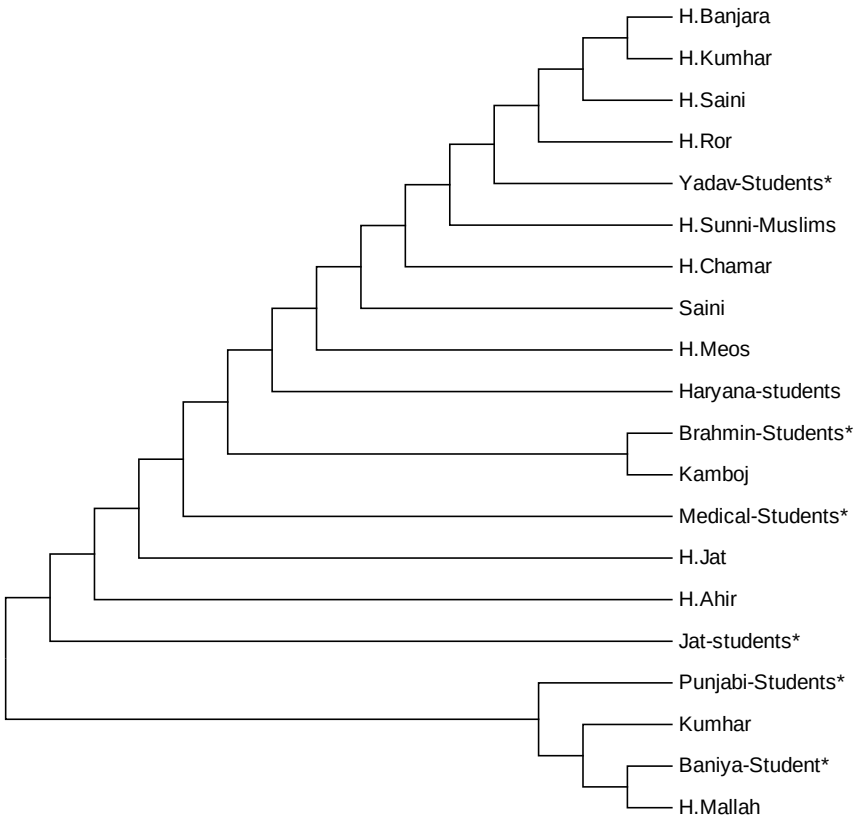

Figure 3. Evolutionary Relationships of 20 Taxa of the Current and Previously Studied Haryana Populations

ABO system plays an important role in transplantation, hereditary diseases, genetic studies and in determining migration of races. Therefore, for the purpose of investigating the pattern of clustering and affinity with 15 previously studied populations from that of other states of India, phylogenetic trees were constructed (Figure 4). The average heterozygosity values ranged from 0.4823 (Andhra Pradesh) (Roychoudhury*et al*., 1992) to 0.66662 (Banjara community of Haryana(Jaggi& Yadav, 2014)). The Jat students were clustered with the previously studied Jat population (Yadav *et al.*, 1997). But the Yadav community from past study was distanced from the Yadav students of the current study. In contrast to this, it is interesting to note that Yadav students under study have shown consistently high genetic distances with other population groups and was clustered in second row.This spectrum difference for Yadav students from other communities may be attributedto sampling error, genetic factors, naturalselection which is affected mainly by traditionsand habits (exogamy, endogamy). The comparison of data study related to the other Indian populations had shown a close relationship between Brahmins of Haryana and other North Indian Brahmins (Yadav *et al*, 2011), pointing towards a common ancestry of these populations.

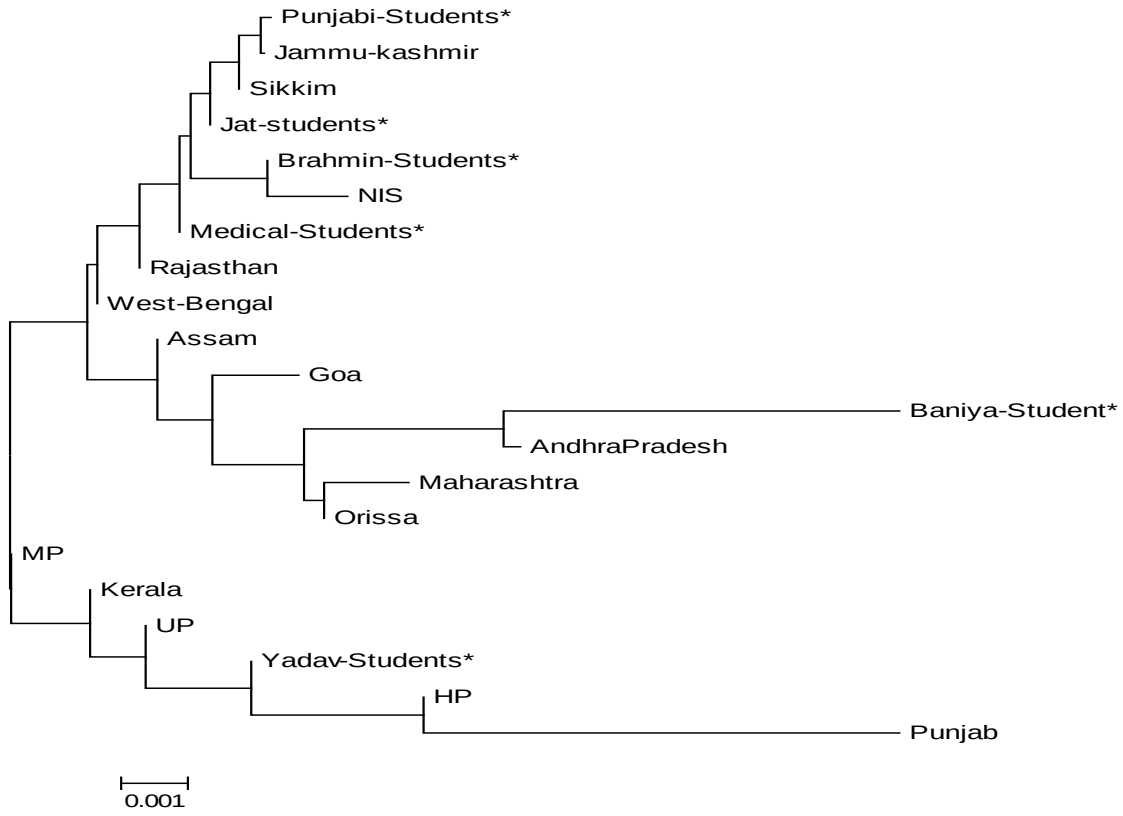

Figure 4. Evolutionary relationships of studied group with Indian population
The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.04306261 is shown. Evolutionary analyses were conducted in MEGA6.

In the analysis of data for Rh (D) factor, 92.632% of the students were observed to be the Rh+ve and 7.368% Rh-ve; the ratio being 92.946% and 7.0539 (for Haryana students). Further observations indicate that, Rhesus factor was comparatively higher in females as compared to males. The high Rhesus negative in the female population is considered adebilitating issue because of the complexity of Rhesus negative during pregnancy and childbirth phase of life (Gunston& Botha, 1980).

CONCLUSION

This study has provided additional data which wouldassist in finding the prevalence of blood group according to the communities; however, fresh data will have to be compiled to make for changes in inter-caste marriages which is now the current trend.The association of different blood groups with diseases has been established, as some of the blood groups are particularly prone to developing certain diseases. The study confirmed that blood group ABO*B was the commonest of the ABO blood group system in the population studied (Haryana), while AB blood group was the least. In addition, comparatively high Rhesus negative in female students warrantsenhanced medical care in the marital life of such students.

The knowledge of the distribution of ABO and Rh factor in blood groups in the medical students will be immense use in health care planning, allocation of resources and targeting the population that need counselling. Such information, well managed can make a major difference in the quality of decisions that individuals can make especially with regards to marriage, blood transfusion and other medical issues.

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