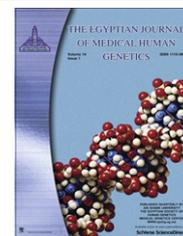




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ORIGINAL ARTICLE

Prevalence and gene frequencies of A_1A_2BO and Rh(D) blood group alleles among some Muslim populations of North India

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KEYWORDS

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Abstract *Background:* Research on ABO group system has been of immense interest, due to its medical importance in different diseases. Till date only a few studies have been done on the prevalence and gene frequencies of A_1A_2BO and Rh(D) blood groups among the Muslim populations of Uttar Pradesh, North India. The data generated in the present work may be useful for health planners while making efforts to face the future health challenges in the region.

Aim: This study was conducted to determine the prevalence and gene frequencies of A_1A_2BO and Rh(D) blood groups among six Muslim populations of Aligarh district, Uttar Pradesh, North India.

Subjects and methods: Blood samples from a total of 724 healthy, unrelated individuals were drawn at random from the six different endogamous groups of Muslim populations of Uttar Pradesh, North India. A_1A_2BO and Rh blood grouping were carried out by standard slide agglutination method and allele frequencies were determined.

Results: In total 724 samples analyzed, the most frequent blood group was found to be group O 29.97% ($n = 217$), followed by A_1 26.52% ($n = 192$), B 20.03% ($n = 145$), A_1B 19.34% ($n = 140$), A_2 2.90% ($n = 21$) and A_2B 1.24% ($n = 9$). The overall phenotypic frequencies of A_1A_2BO blood groups were $O > A_1 > B > A_1B > A_2 > A_2B$. The calculated allelic frequencies were 0.5619, 0.2214, 0.1973 and 0.0259 for group I^O , I^B , I^{A1} and I^{A2} , respectively. The Chi-square differences for A_1A_2BO blood groups among different Muslim populations were found to be significant

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($\chi^2 = 41.22$, $df = 25$, $p < 0.02$). Out of total 724 samples, 613 (84.67%) samples were Rh + ve and 111 (15.33%) were Rh - ve.

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1. Introduction

India, home to approximately one-fifth of the total world population, is known for its sociocultural, religious and genetic diversity [1]. The rich genetic diversity of Indian populations is unique and offers excellent opportunities for genetic and anthropological researches. The country has witnessed successive waves of migration since prehistoric times resulting in fresh gene flow, besides adding their customs, languages and cultures. These new social and cultural strains have made the population of India diverse and heterogeneous. The reproductive isolation of these populations though varied is mainly geographic, religious, linguistic, ethnic or occupational. As natural selection works on the whole individual, heritable traits will become more common in the next generation. Given enough time, this passive process can result in progressive adaptation and speciation [2]. The affinities of Indian populations to other world populations, including ancestral groups, should provide valuable information on the origin of our species. In addition, India's strategic location as a migratory crossroad may allow us to ascertain the paths of several major human dispersals [3].

Among the various known religions found in India (Islam, Christianity, Sikhism, Buddhism, Jainism and Judaism), Islam is the second most practiced religion, after Hinduism. Islam reached Indian shore in the 7th century anno domini (AD). Most of the Muslims belong to Indian ethnic groups with very minor levels of gene flow from outside, primarily from Persia and Central Asia. Central Asian Muslims moved into India in the end of tenth century from the northwest and expanded throughout the sub-continent.

In North India the state of Uttar Pradesh is historically important because of its old centers of population, learning and administration. It is the fifth largest state of India with an area of 243,286 km². Because of Islamic influence for centuries, Uttar Pradesh becomes the heartland of Indian Muslims, becoming home to 32 million Muslims, nearly one-third of Indian Muslim total population [4].

The Aligarh city in Uttar Pradesh is situated between latitude 27.28° to 28.10° North 77.29° to 78.36° east longitude and its total area is 34.05 km². Aligarh has almost a dry climate throughout the year. The annual average rainfall in the district is 594.1 mms and maximum temperature recorded is 44 °C. The population of Aligarh is composed of Hindus, Muslims and Christians. Hindus comprise Brahmins, Jats, Baniyas, Thakurs and Balmikis while Muslims comprise Syed, Sheikh, Mughal, Pathan, Shia, Sherwani, Ansari and Saifi.

Serological markers have served as important indicators for the understanding of genetic variation between and within populations. Blood groups are the simple and useful tool and can be used for the purpose. Of all the genetic characters, blood groups have been exclusively employed in the study of genetic structure of human populations all over the world. The blood group of an individual does not change in its lifetime and hence, acts as a unique genetic marker for research.

Since 1901, more than 20 distinct blood group systems have been characterized but the ABO and Rhesus (Rh) blood groups remain the most clinically important. Both these systems are useful in blood transfusion and organ transplantation. They are also well-defined genetic markers employed in population genetic and anthropological studies [5,6].

The distribution of these two blood groups has been repeatedly investigated in various populations all over the world during the past half-century. The frequencies exhibit considerable variation in different geographic locations, reflecting the underlying genetic and ethnic diversity of human populations [7].

ABO blood grouping system was established by Karl Landsteiner in 1900 [8] on the basis of the presence or absence of two antigens (A and B) on RBC and its Mendelian inheritance pattern by Bernstein in 1924 [9]. In this system, four blood groups namely A, B, AB and O are identified by blood tests. The fourth blood type (AB) was discovered by DesCasterllo and Sturli in 1902 [10]. These antigens are under control of three allelic genes, namely *IA*, *IB* and *io* which determine blood groups. *IA* produces A antigen, *IB* produces B antigen whereas *io* produces neither. *IA* and *IB* are mutant alleles and show codominance with each other but both are dominant over the wild type allele *io* [11]. ABO antigens are one of the oligosaccharides antigens [12]. These antigens are widely expressed on the membranes of red cell and tissue cell as well as, in the saliva and body fluid [13]. The ABO locus is located on chromosome 9 at 9p34.1–q34.2 and encodes glycosyltransferases.

The Rh blood group, popularly referred to as Rhesus, is second only to the ABO system in its importance in transfusion medicine. The presence of Rh system was recognized in 1939 that was confirmed within few years. Although the Rh system is highly polymorphic, and comprises at least 44 distinct antigens, clinically the most significant polymorphism is due to the presence or absence of the Rh(D) antigen on red cells.

Research on ABO group system has been of immense interest, due to its medical importance in different diseases. The ABO blood groups system is not only important in blood transfusions, cardiovascular diseases, organ transplantation, erythroblastosis in neonates, but also one of the strongest predictors of national suicide rate and a genetic marker of obesity [14–19].

The studies on the genetic markers among Muslim population are very few, therefore an attempt has been made to study blood groups A₁A₂BO and Rh(D) distribution among some Muslim populations of Uttar Pradesh, North India.

2. Subjects and methods

2.1. Populations

The Muslims of India comprise more than 13% of the population, and they belong to various castes, based on linguistic and

ethnic groups besides, having a few tribes [20]. Muslims belong to two major sects: Sunnis and Shias, while each sect has different biradaris, which are grouped under Ashraf and Ajlaf [21]. The former comprise higher rank Muslims like Syeds, Sheikhs, Pathans and Mughuls while the latter comprise Qureshis, Ansaris, and Saifis. A large number of the Ajlaf may also be converted from local indigenous population of other faiths [22].

The other Muslim castes are represented by the Julaha, Nai, Darzi (Tailor), Kasai (Butcher), Mirasi (Musician), Barhai (Carpenter), Dhobi (Washerman), Kumhar (Potter), Teli (Oil-presser) and Lohar (Blacksmith). The Saifis are a Muslim community found in North India. It is said that those who manufactured swords in past were known as Saifi, because the word *saif* in Arabic means “sword”. They are traditionally carpenters and blacksmiths. Saifis are world’s first engineers.

2.2. Sample collection

Blood samples were collected by finger-pricks from seven hundred and twenty-four (724) healthy, unrelated individuals of both sexes belonging to Syed, Sheikh, Pathan, Shia, Sherwani and Ansari populations of Aligarh District, Uttar Pradesh, North India.

The survey was conducted among healthy individuals during November 2011 to February 2012 for A₁A₂BO and Rh(D) loci. Households were selected through door to door contact by the investigator. Each subject was interviewed before screening. His/her general particulars (address, age, sex, ethnic group) were recorded. Information regarding previous transfusion or blood donation was also obtained. Fingertip blood was routinely used for grouping. The samples were collected from the Upper Court, Civil Lines, Hamdard Nagar, Friends Colony, AMU Campus, Sir Syed Nagar and Jamalpur areas of Aligarh.

2.3. Laboratory analysis

ABO and Rh-D grouping were performed simultaneously. Slide agglutination method was followed. On a labeled slide a drop of anti-A, anti-B and anti-D was placed and a drop of finger-prick blood was added to each and was mixed. Results of agglutination were recorded immediately. Agglutination with anti A, showed A group, with anti-B showed the B group, with both A and B, it showed AB and with neither of these showed O groups [23]. The gene frequencies for these two systems were calculated after Mourant et al. [24]. Chi-square test was applied for statistical analysis.

2.3.1. Subgroups of ABO

Subgroups are classified by the quantity of A antigen, and the amount of A antigen decreases in the order A₁, A₂, A₃, A_x, A_{end}, A_m, A_{el}. In Europeans, approximately 80% of blood type A and AB belong to A₁, the remaining 20% are either A₂ or A₂B (in Japanese it is approximately 0.2%) [25,26]. Anti-A₁, lectin is a purified extract of the seeds of *Dolichos biflorus* containing phytohemagglutinin(lectin) which agglutinates human red blood cells only of the subgroup A₁ or A₁B. Blood collected with or without an anticoagulant was used in the testing procedure. Agglutination is a positive test result for the presence of human blood groups A₁ and A₁B. The weaker subgroups of A (A₂, A₃, A₂B, A₃B, etc.) are not agglutinated.

2.3.2. Rh(Anti-D Anti-Rho test method)

A blood sample is classified as Rh-positive or Rh-negative according to the presence or absence of the antigen D. Screening for the Rh typing may be conducted by the slide method. Agglutination of the red blood cells in the slide constituted a positive test result of the D antigen.

2.4. Statistical analysis

Phenotypes were recorded for each trait and gene frequencies were calculated according to Hardy–Weinberg law [27] using the gene counting method. Heterozygosity for a given locus was calculated using the genotype frequencies (for heterozygous genotype). The level of heterozygosity was calculated using the formula:

$$\text{Heterozygosity} = 1 - \sum H_o$$

where H_o is the homozygosity of the allele, $H_o = \sum Pi^2$.

Chi-square test: It is used for the measurement of the size of the discrepancy between the observed and expected values at particular degrees of freedom at 5% level of probability:

$$\chi^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

Yates correction has also been done wherever needed.

3. Results

The results of the distribution of A₁A₂BO blood groups and Rh(D) phenotypes and gene frequencies among the Muslim populations of UP have been presented.

3.1. Phenotypic frequency

3.1.1. For A₁A₂BO

In total 724 samples analyzed, blood group O was the most prevalent (29.97%), followed by groups A₁ (26.52%), B (20.03%), A₁B (19.34%), A₂ (2.90%) and the smallest one is A₂B (1.24%). The overall phenotypic frequencies of A₁A₂BO blood groups follow the trend, i.e. O > A₁ > B > A₁B > A₂ > A₂B. Phenotypically O group was dominant and A₂B was rare among all population groups. The χ^2 differences were significant only in Shia population group and remaining populations showed non-significant differences ($\chi^2 = 16.96$, df = 5, $p > 0.005$). The overall Chi-square (χ^2) values for A₁A₂BO blood groups among different Muslim populations were found to be statistically significant ($\chi^2 = 41.22$, df = 25, $p < 0.02$) (Table 1 and Fig. 1). Yates correction was done in case of small samples (lower than 5).

3.1.2. For Rh

Out of the 724 subjects tested 613 (84.67%) subjects were Rh+ve and 111 (15.33%) subjects were Rh–ve. The highest of Rh–ve individuals is found in Sheikh (19.09%), while in other populations it ranges from 11to19%. The χ^2 values were significant among Syed, Sheikh and Pathan population groups ($\chi^2 = 1.43$, df = 1, $p < 0.20$ for Syed; $\chi^2 = 1.20$, df = 1, $p < 0.20$ for Sheikh; $\chi^2 = 1.52$, df = 1, $p < 0.02$). The overall Chi-square (χ^2) values for Rh(D) blood groups among different Muslim populations were found to be statistically insignificant ($\chi^2 = 5.2705$, df = 5, $p = 0.3837$) (Table 2).

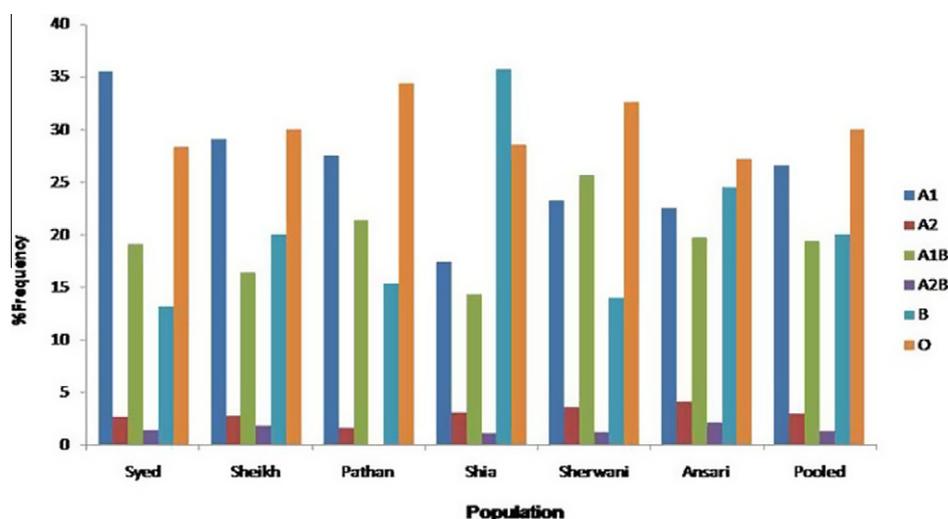
Table 1 Phenotype frequencies of A₁A₂BO marker loci for different Muslim populations of Uttar Pradesh, North India.

Populations (No.)	A ₁ No. (%)	A ₂ No. (%)	A ₁ B No. (%)	A ₂ B No. (%)	B No. (%)	O No. (%)
Syed (152)	54(35.53)	4(2.63)	29(19.08)	2(1.31)	20(13.16)	43(28.29)
Sheikh (110)	32(29.09)	3(2.73)	18(16.36)	2(1.82)	22(20.00)	33(30.00)
Pathan (131)	36(27.48)	2(1.53)	28(21.37)	0(0.00)	20(15.27)	45(34.35)
Shia (98)	17(17.35)	3(3.06)	14(14.29)	1(1.02)	35(35.71)	28(28.57)
Sherwani (86)	20(23.26)	3(3.49)	22(25.58)	1(1.16)	12(13.95)	28(32.56)
Ansari (147)	33(22.45)	6(4.08)	29(19.73)	3(2.04)	36(24.49)	40(27.21)
T ± SE (724)	192(26.52) ± 1.6	21(2.90) ± 0.6	140(19.34) ± 1.5	9(1.24) ± 0.4	145(20.03) ± 1.5	217(29.97) ± 1.7

Parentheses = percentage (%) and No. = number of individuals.

$\chi^2 = 41.22$, $df = 25$, $p < 0.02$ (statistically significant).

T = total and SE = standard error.

**Figure 1** Graph showing Phenotype frequencies of A₁A₂BO marker loci for different Muslim populations of North India.**Table 2** Phenotype frequencies of Rh marker loci for different Muslim populations of Uttar Pradesh, North India.

Populations	No.	Rh(+ve) No. (%)	Rh(-ve) No. (%)
Syed	152	134(88.16)	18(11.84)
Sheikh	110	89(80.91)	21(19.09)
Pathan	131	116(88.55)	15(11.45)
Shia	98	81(82.65)	17(17.35)
Sherwani	86	70(81.39)	16(18.60)
Ansari	147	123(83.67)	24(16.33)
T ± SE	724	613(84.67) ± 1.3	111(15.33) ± 1.3

Parentheses = percentage (%) and No. = number of individuals.

$\chi^2 = 5.2705$, $df = 5$, $p = 0.3837$ (statistically insignificant).

T = total and SE = standard error.

3.2. Allele frequencies

3.2.1. For A₁A₂BO

Table 3 presents distribution of the alleles in the total sample and was recorded 0.5619, 0.2214, 0.1973 and 0.0259 for I^o, I^b, I^{a1} and I^{a2}, respectively indicating that the allelic frequency of I^o is the highest, while I^{a2} is the lowest. All the populations follow the trend I^o > I^b > I^{a1} > I^{a2}. Pathan showed the highest I^o and lowest I^{a2} frequency. The Ansaris have the highest I^{a2} allele frequency, may be due to a lower population group.

3.2.2. For Rh

It shows the allele frequency of D is 0.6085 and that of d is 0.3915. D allele has the highest frequency in Pathan and lowest in Sheikh (Table 4 and Fig. 2).

3.3. Heterozygosity

The pooled heterozygosity for A₁A₂BO is found to be 0.5985 and for Rh it is 0.4765. For A₁A₂BO, heterozygosity ranges from 0.5697 to 0.6128 and for Rh system it varies from 0.4478 to 0.4920 (Table 5 and Fig. 3).

4. Discussion

The present study is useful in providing information on the status of blood group marker in the Muslim populations of Uttar Pradesh. The data generated in the present work not only provide the availability of human blood but also serve to enable insight into possibilities of future burden of diseases. Blood groups and Rh antigen are hereditary. Gene for ABO antigens is on chromosome 9 and for Rh antigen is on the chromosome 1 [28]. The information of blood groups is very useful in blood transfusion and organ transplantation medicine, in human population migration and evolution study, in genetic research and in parental dispute cases. It is, therefore, imperative to

Table 3 Allele frequencies of A₁A₂BO marker loci for different Muslim populations of Uttar Pradesh, North India.

Populations	I ^{a1}	I ^{a2}	I ^b	I ^o
Syed	0.2592	0.0241	0.1781	0.5476
Sheikh	0.2142	0.0244	0.2079	0.5592
Pathan	0.1970	0.0128	0.1953	0.6020
Shia	0.1375	0.0279	0.2952	0.5419
Sherwani	0.1697	0.0298	0.2163	0.5925
Ansari	0.1737	0.0378	0.2576	0.5370
T ± SE	0.1973 ± 0.015	0.0259 ± 0.006	0.2214 ± 0.015	0.5619 ± 0.018

I^{a1}, I^{a2}, I^b and I^o are allele frequencies of blood group A₁, A₂, B and O, respectively.

T = total and SE = standard error.

Table 4 Allele frequencies of Rh marker loci for different Muslim populations of Uttar Pradesh, North India.

Populations	D	d
Syed	0.6559	0.3441
Sheikh	0.5631	0.4369
Pathan	0.6616	0.3384
Shia	0.5835	0.4165
Sherwani	0.5687	0.4313
Ansari	0.5959	0.4041
T ± SE	0.6085 ± 0.018	0.3915 ± 0.018

T = total and SE = standard error.

D and d are dominant and recessive alleles, respectively.

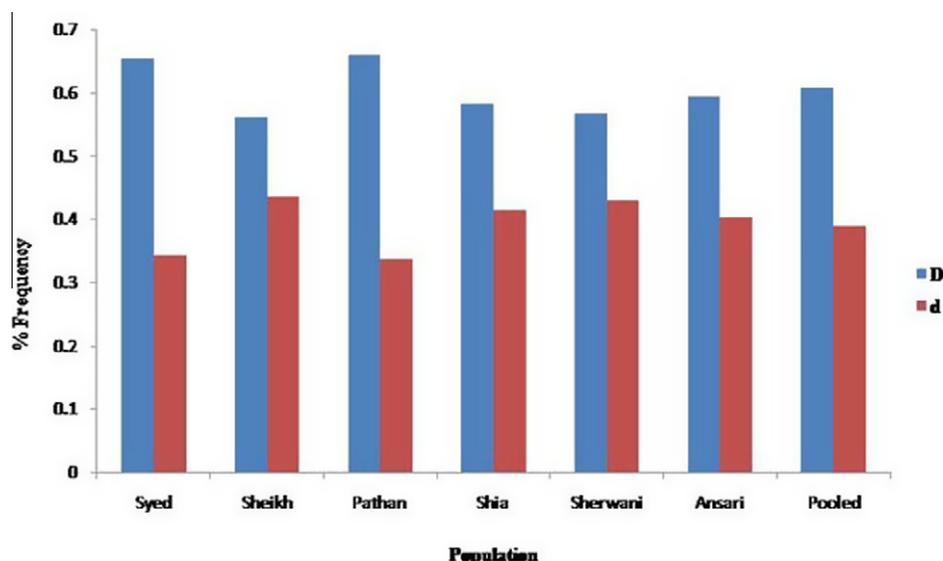
Table 5 Heterozygosity at A₁A₂BO and Rh marker loci for different Muslim populations of Uttar Pradesh, North India.

Populations	A ₁ A ₂ BO H _t	Rh H _t
Syed	0.6062	0.4514
Sheikh	0.5986	0.4920
Pathan	0.5697	0.4478
Shia	0.5969	0.4861
Sherwani	0.5790	0.4906
Ansari	0.6128	0.4816
Total	0.5985	0.4765

H_t represents heterozygosity.

have information on the distribution of these blood groups in any population group. Few studies of ABO and Rh blood group prevalence among the various populations of India have been carried out. Some studies on Muslim populations have been attempted earlier in Uttar Pradesh. These are described here for comparison. Our studies show some differences from earlier studies on Muslim populations of North India. The endogamous groups of Muslims in different regions are heterogeneous with respect to the gene frequencies of ABO blood groups [29,30]. There are significant differences in allelic frequency between different subgroups for this marker.

During the last five decades numerous studies have been carried out on the genetic composition of various Muslim population groups in India [31–33]. Our study is comparable to the ABO frequencies reported in some earlier studies of other caste groups of UP. Majumdar (1943) [34] reported the distribution of ABO blood groups in the Shia Muslim populations of Jaunpur in which the percentage of different types reported were B, 34%, O, 36%, A, 25% and AB, 5%. The overall picture for Sunni Muslim from Madhya Pradesh has the following frequencies: A, 19.1%, B, 22.4%, and O, 58.4% [35].

**Figure 2** Graph showing Allele frequencies of Rh marker loci for different Muslim populations of North India.

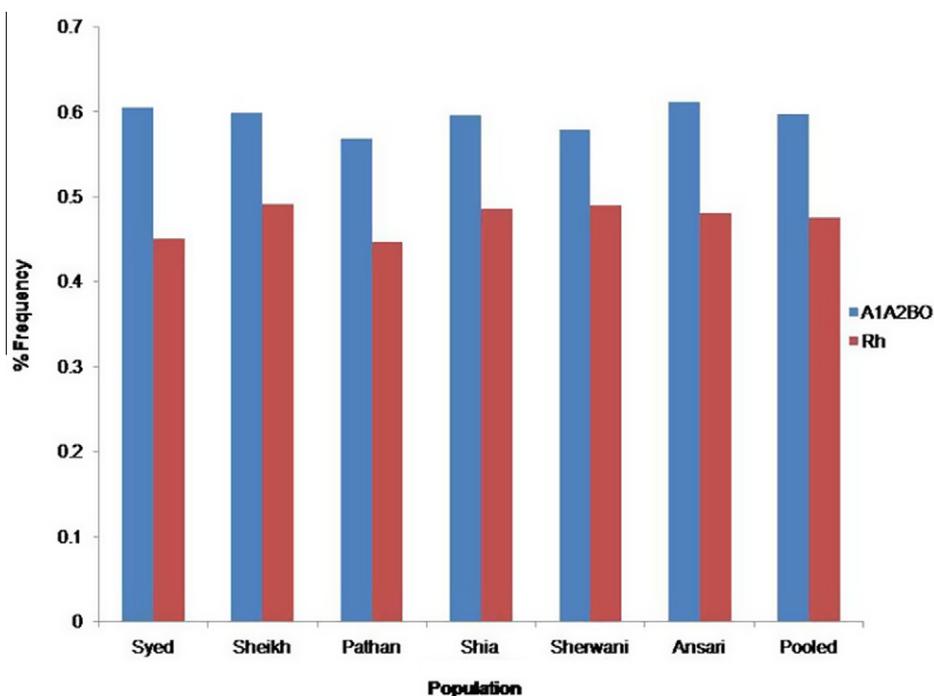


Figure 3 Graph showing Heterozygosity at A_1A_2BO and Rh marker loci for different Muslim populations of North India.

The distribution of ABO blood groups varies regionally, ethnically and from one population to another. From this study, the distribution of blood group O was the highest with percentage frequency of 29.97% followed by blood group A_1 26.52%, B 20.03%, A_1B 19.34%, and A_2 2.90% and the least percentage frequency is that of blood group A_2B which is 1.24%.

Normally, the distribution of ABO blood groups varies from one population to another. In many other studies, blood group O has been found to be the most common blood group. In the Caucasians in the United States, the distribution is group O, 47%, group A, 41%, group B, 9% and group AB, 3% [36]. Among Western Europeans 42% are group A, 9% group B, 3% group AB and the remaining 46% group O. For blacks in United States, the distribution is group O, 46%, group A, 27%, group B, 2%, and group AB, 7% [36]. Similarly, in Pakistan, blood group O is the most common 35%, blood group A is 24%, blood group B is 33% and blood group AB is 8%. In Lagos Nigeria, blood group O is 55.3%, blood group A, 25.3%, blood group B, 16.7% and blood group AB, 2.7% [37]. Iyiola et al. [38], also reported phenotypic frequencies of 52.9%, 23.1%, 21.3% and 2.7% in the order $O > A > B > AB$. We observed that the most available data from Nigeria and some parts of the world reported the proportions of A, B, AB and O in the order $O > B > A > AB$ [39,40].

The frequency of ABO blood groups varies from race to race. The country wise figure in Caucasians for the United States, the distribution is type O = 47%, type A = 41%, type B = 9%, and type AB = 3%. Among African American, the distribution is type O = 46%, type A = 27%, type B = 20%, and type AB = 7%. Among Western Europeans, 42% population shows blood group A, 9% blood group B, 3% blood group AB and the remaining 46% blood group O [41]. The allelic frequencies of the total population of the world

are found to be O = 62.3%, A = 21.5% and B = 16.2%. American blacks generally have frequencies of A, B, AB and O blood groups of 27%, 20%, 4%, and 49%, respectively [42].

For A_1A_2BO blood groups, no more studies have been done on Muslim populations but Ara et al. [43], reported the distribution of ABO subtypes in different Muslim and Hindu populations of UP in which, the phenotypes have the following frequencies: O = 30.69%, A_1 = 24.64%, A_1B = 20.21%, B = 18.88%, A_2 = 3.97% and A_2B = 1.60% which are very close to our values. In India, the distribution of allele B frequency is higher (23.3%) as compared to allele A (18.6%), whereas the frequency of allele O is (58.1%). In present study the pooled frequencies of allele O = 56.1%, B = 22.1%, A_1 = 19.7% and A_2 = 2.5% which are nearly similar as compared to studies reported on different Muslim populations of UP by Ara et al. [43].

The Rh distribution also varies within any group of population. The recessive allele (d) ranges from as high as 40% to its virtual absence in Chinese Australian aborigines, Negrito etc. Exceptionally high incidence of Rh negatives yielding frequency of recessive allele (d) in the range of 50–60% has been reported in Basque (Europe) and Berbers of Morocco [24].

The variation in frequency of the Rh negative gene (i.e., Rh d) is 15–30% in the majority of the India population, compared to 35–45% in Europeans and 0–10% in Asian population [44]. For the Rh system the overall frequencies for D and d are 80.71% and 19.29%, respectively [45], which compared to 60.85% and 39.15% in our case. Several studies reported on Rh system in North India [31].

The principal bio-clinical significance of the erythrocytic group antigens is still associated with the living immune characteristics. It plays a special role in blood transfusion [46], epidemiology [47] and transplantology [48,49]. In view of the importance of blood groups in population characteristics, the

present study is done to investigate the frequency of ABO, subgroup ABO and Rh(D) in some Muslim populations of Uttar Pradesh (UP), North India.

5. Conclusion

The present study shows the prevalence of blood group marker among six Muslim populations of Aligarh district, UP. In this study it can be seen that the most frequent blood group is O and the A₂B has the least percentage. The Rh-ve frequency is 15%. The Chi-square differences revealed that the prevalence of A₁A₂BO blood groups among different Muslim populations had been significant. The study has a significant implication regarding the management of blood bank and transfusion services in this area. Knowledge of the blood group system helps us to take preventive measures for the diseases which are associated with different blood groups, to prevent the dangerous transfusion reactions and efficient management of regional blood bank and transfusion services.

Conflict of interest

The authors declare that there is no conflict of interest.

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