

Research Article

Gene frequency distribution of ABO and Rh-D blood group alleles in Multan Division (Punjab), Pakistan

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Abstract

This study was conducted during 2017-19 with the objective to provide data of distribution of blood group of ABO and Rh(D) and gene frequency among the population of three districts; Multan, Lodhran and Khanewal (Multan Division), Pakistan. This information will provide basic facts and figures of region to geneticists, practitioners and blood transfusion programmers. From both genders total 440 subjects were selected from schools and colleges students, prisoners and factory workers etc. randomly in different regions of three districts. Blood samples of both genders were tested for blood groups ABO and Rh(D) factor with help of open slide test method. To observe agglutination, a drop of the antisera, anti-A, anti-B and anti-D were mixed with every blood sample and shook gently for 60 seconds. The predominant blood group was O with 36.48%, 42.57% and 42.08% presence in Multan, Lodhran and Khanewal respectively in all the Rh(D) positive subjects. In Rh(D) negative subjects, blood group A was found predominant with 5.40% in Multan district only amongst the three districts. In Multan the percentage of Rh(D) Positive was 90.55% and Rh(D) negative was 9.45%. In Lodhran, the percentage of Rh(D) positive was 93.25% and Rh(D) negative was 6.75%. in Khanewal, the percentage of Rh(D) positive was 91.22% and Rh(D) negative was 8.78%. Among the 3 districts studied, both Rh(D) positive and Rh(D) negative frequency of blood groups was O > A > B > AB > except Multan where blood group "A" was common among Rh(D) negative subjects.

Keywords: ABO; Distribution; District; Division; Frequency; Population; Rh(D)

Introduction

Blood is known as the very important constituent of body fluid besides the most critical type substance like oxygen for the

circulation of valuable hormones, nutrients and enzymes to across all parts of the body. The Australian scientist Karl Landsteiner in 1900 discovered the ABO blood system

(three different blood systems) with three different blood types categorized as A, B and O [1]. Furthermore, ABO blood system was classified into A, B, AB and O blood groups based on the surface of the red blood cells. Rhesus-negative (Rh) and Rhesus-positive (Rh) in Rhesus (Rh) type system which is based on the presence or absence of the inherited antigenic substances. Depending on the blood group system the antigens may be glycolipids, glycoproteins, carbohydrates and proteins [2]. In a mandelian fashion development of all these types of antigens are controlled through genetically and appear early in the fetal life while remain unchanged till death [3]. By the International Society of Blood Transfusion, nearly 750 erythrocyte antigens are described and organized into 45 blood group systems of which ABO and the types Rh are most valuables [4].

The ABO and Rh blood groups besides the practice of blood transfusion are also of critical importance regarding genetic studies, disputed paternity cases, medico-legal issues and studying population migration patten, etc. [5]. This ABO blood system is shared by all human population but may differ in frequency of particular types. This incidence of variation of ABO and Rh groups in different areas of the world may found among races, socio-economic and of ethnic groups [6]. For safe blood transfusions and effective blood bank services, the knowledge of this ABO type and the type of Rh blood group distribution at local and as well as regional levels is very important [7]. There are multipurpose of estimating gene frequency and blood groups as it provides precious information about genetic similarities of different types of populations, ancestral genetic linkages and to some extent the religious and cultural differences between the various populations [8]. Due to racial differences, these are present even in Pakistan [9]. In transfusion of blood, organ transplantation, evolution and genetic

research the blood groups prevalence plays an important role. With environment modern types of medicine is also working on due to relationship of the blood group [10].

The valuable aim of the present study was considered to find out the frequency of all these blood groups of ABO and the Rh(D) in three districts of Multan Division. Another purpose of this study was to produce data for multipurpose future types of utilities.

Materials and Methods

Total of 444 ABO and the Rh(D) blood samples of both genders from different populations like schools, colleges, prisons and factories were collected from Multan, Lodhran and Khanewal (3 different regions of Multan Division) Punjab, Pakistan. The samples were selected from the donor records of blood donation camps of these areas based on 02 years (2017-2019) records and prior to collection of samples, consents were granted. With the use of sterile lancet and finger prick, without any anti-coagulant, the blood samples were collected on open slide method of the ABO and the Rh(D) blood groups [11]. Before pricking, the bulb was sterilized with alcohol. After slightly pricking, compressed lancet, the oozed type of blood drop were placed on a transparent glass slide at 3 (three) different spots. To each blood drop on a transparent glass slide, a drop of the anti-A, anti-B and anti-D (anti-sera, prepared by Biolaboratory, USA) were added and then separately observed for the agglutination. This agglutination test helped to identify blood grouping by antigen-antibody. The frequencies expressed as in different percentages were calculated, Chi-square test for the distribution of blood group and Rh antigen were also carried under the valuable assumption of Hardy-Weinberg equilibrium.

Results

The four hundred and forty four (444) school and college students, prisoners in jail and factory workers etc were selected randomly. This sample consist of 257 males and 187

females types between ages 16 and 52 years. The frequency type distribution of the blood group (ABO) differs among the studied populations of these three districts of Multan Division. The highest frequency was of “O” blood group and the “AB” blood group gained the lowest type of frequency (Table 1). Blood group “O” was highly in distribution in Khanewal as compared to Multan and Lodhran districts, whereas the frequency found to be in blood group “A” was high in Multan District than that of Lodhran and Khanewal Districts. Equal frequency was found of “AB” blood group in all three studied districts.

The frequency distribution of type Rh-positive and the Rh-negative variations followed the same pattern amongst the three district groups followed by the similar method is shown in (Table 2). Based on the Rh type of blood group the percentage (%) in distribution of the ABO type blood groups and the district groups varies significantly (Table 3). The distribution of ABO type blood group type based on the Rh in the population of Multan and Lodhran districts is

almost the similar in the blood group type A with that of Rh type positive (27.03%) but in Khanewal district the %age of the blood group type A is to reduce to 31 (20.95%) of the known total population. In the three district groups the blood of group AB with that of Rh positive (+) has a small % (percentage) in distribution than the blood type group A and B (Table 3). In population of Multan district blood type of group O with the Rh positive (+) was 53 (35.82%) as compared to that of Lodhran district population was 60 (40.55%) which is higher than the Multan district population and 68 (45.95%) for the Khanewal district population. So, O blood group which is with Rh type of positive is known to be dominant in Khanewal district population. In this three districts group of population, Blood group “O” with Rh type of positive (+) varies significantly in distribution as compared to other types of blood groups. However, in the three representative groups of population the percentage in the type of distribution of type Rh negative (-) which is very rare or small (Table 3).

Table 1. Phenotypics distribution of the ABO type of blood group system amongst the Population of three district groups at Multan Division during 2017-2019

District groups	Frequency distribution of blood type				Total
	Blood Type A	Blood Type B	Blood Type AB	Blood Type O	
Multan	48 (32.44%)	37 (25.00%)	9 (6.08%)	54 (36.48%)	148 (100%)
Lodhran	43 (29.05%)	33 (22.30%)	9 (6.08%)	63 (42.57%)	148 (100%)
Khanewal	36 (24.32%)	30 (20.28%)	9 (6.08%)	73 (49.32%)	148 (100%)
Total	127 (28.60%)	100 (22.52%)	27 (6.08%)	190 (42.80%)	444 (100%)

Table 2. Phenotypic type of distribution of Rhesus (Rh) factor of blood group amongst the population of three district groups at Multan Division during 2017-2019

District Groups	Rhesus (Rh) system		Total
	Rh positive	Rh negative	
Multan	134 (90.55%)	14 (9.45%)	148 (100%)
Lodhran	138 (93.25%)	10 (6.75%)	148 (100%)
Khanewal	135 (91.22%)	13 (8.78%)	148 (100%)
Total	407 (91.66%)	37 (8.34%)	444 (100%)

Table 3. ABO types of blood group frequency distribution based on Rh blood group of system amongst the population of three district groups at Multan Division during 2017-2019

District group	Rhesus system	A	B	AB	O	Total
Multan	Positive	40 (27.03%)	33 (22.29%)	8 (5.40%)	53 (35.82%)	134 (90.54%)
	Negative	8 (5.40%)	4 (2.70%)	1 (0.67%)	1 (0.67%)	14 (9.45%)
Lodhran	Positive	40 (27.03%)	32 (21.63%)	6 (4.05%)	60 (40.55%)	138 (93.25%)
	Negative	3 (2.02%)	2 (1.35%)	1 (0.67%)	4 (2.70%)	10 (6.75%)
Khanewal	Positive	31 (20.95%)	28 (18.92%)	8 (5.40%)	68 (45.95%)	135 (91.22%)
	Negative	5 (3.37%)	2 (1.35%)	1 (0.67%)	5 (3.37%)	13 (8.78%)
Total	Positive	111 (25.00%)	93 (20.94%)	22 (4.96%)	181 (40.76%)	407 (91.66%)
	Negative	16 (3.06%)	8 (1.80%)	1 (0.22%)	10 (2.25%)	37 (8.34%)

Among the population of each district group the overall allelic frequencies as it was calculated with the help of extension of the Hardy-Weinberg Equilibrium were 0.66, 0.200 and 0.15 for types of O, A and B alleles respectively (Table 4). Following rhesus status, 93.07% were Rh(D) positive while 6.93% were Rh(D) negative. This allele gave the different allelic types of frequencies considered as 0.73 for allele D and 0.27 for allele d respectively.

The (Table 4) showed comparison between different observed types and expected types values for both ABO types of blood group and the Rh(D) factor of all three district groups respectively. The observed known frequency in this population was to be found of 91%, 94% and 93% and the expected frequency was 96.3% for Multan, Lodhran and Khanewal groups accordingly, while the observed known frequency of the Rh(D) types negative (-) was 9%, 8% and 8% for Multan, Lodhran and Khanewal respectively while expected frequency was 6.2%.

We found that under Hardy Weinberg Equilibrium the distribution of and the proportion of different individual types

having ABO types of blood group antigens which did not show difference from those as compared to expected frequency for Multan and Lodhran districts groups (goodness-of-fit X^2 for ABO=1.52, df=2, $P<0.05$; goodness-of-fit X^2 for ABO=0.382, df=2, $P<0.05$) which is statistically known as insignificant. However, the distribution of and the proportion of different individuals which having ABO types of blood group antigens under the Hardy Weinberg Equilibrium differ from those of expected values for Khanewal district group (goodness-of-fit X^2 for ABO=2.055, df=2, $P<0.05$). The difference is not to be significant. For all the three groups the distribution of and the proportion of the individuals having the Rh(D) blood group antigens did not differ as compared from those of expected values under Hardy Weinberg Equilibrium (goodness-of-fit X^2 for Rh=3.23, df=1, $P>0.05$ for Multan district; goodness-of-fit X^2 for Rh=0.54, df=1, $P>0.05$ for Lodhran district; goodness-of-fit X^2 for Rh=1.52, df=1, $P>0.05$ for Khanewal district; which is statistically considered to be insignificant.

Table 4. Gene frequency distribution of ABO type and Rh (D) blood group types of alleles amongst the population of three district groups at Multan Division during 2017-2019

District group	Gene (allele)	Frequency	Genotype	Frequency	Phenotype	Frequency (%)
Multan	O(r)	0.6062	OO	0.3873	O	38.73
	A(p)	0.2330	AA	0.0488	A	4.88
	B(q)	0.1714	AO	0.2799	A	27.99
			BB	0.0294	B	2.94
			BO	0.2000	B	20.00
			AB	0.0545	AB	5.45
	D	0.7000	DD	0.4900	Rh(D)+ve	49.00
		0.2980	Dd	0.4200	Rh(D)+ve	42.00
Lodhran	d		Dd	0.0900	Rh(D)-ve	9.00
	O(r)	0.6646	OO	0.4286	O	42.86
	A(p)	0.2000	AA	0.0371	A	3.71
	B(q)	0.1563	AO	0.2477	A	24.77
			BB	0.0536	B	5.36
			BO	0.1788	B	17.88
			AB	0.0545	AB	5.45
	D	0.7250	DD	0.5200	Rh(D)+ve	52.00
Khanewal			Dd	0.4200	Rh(D)+ve	42.00
	d		Dd	0.0800	Rh(D)-ve	8.00
	O(r)	0.7048	OO	0.4977	O	49.77
	A(p)	0.1525	AA	0.0242	A	2.42
	B(q)	0.1439	AO	0.2148	A	21.48
			BB	0.0205	B	2.05
			BO	0.1906	B	19.06
			AB	0.0545	AB	5.45
	D		DD	0.5100	Rh(D)+ve	51.00
			Dd	0.4200	Rh(D)+ve	42.00
	d	0.2950	Dd	0.0880	Rh(D)-ve	8.00

Discussion

Throughout the life phenotypes and ABO and Rh type genes differ broadly across all races and the geographical distributions despite the fact of that antigens which are involved known as stable. In population studies, the resultant type of polymorphism remains most valuable in evaluating the accessibility of compatible blood, estimating the chances of hemolytic diseases of newly born babies and determining disputes of maternity / paternity and for purpose of forensic [12, 13]. Therefore, this study is helpful for these three studied districts of Multan regarding distribution status of the ABO and the Rh type of blood groups.

Blood group “O” was highly distributed in Khanewal as compared to Multan and Lodhran districts, whereas the frequency of “A” type blood group was high in Multan District than Lodhran and Khanewal Districts. Blood group “B” was in dominance in Multan District than Lodhran and Khanewal. Equal frequency was found of “AB” blood group in all three studied districts. In connection with our findings previous studies conducted by [14] as well as [15] showed that there are variations among different groups in the blood of ABO group. For the present study gene frequencies which are with concern to ABO blood group system which can be compared with regards to a

general or common formula of $O > B > A > AB$ that does not derived from previous works in different countries in all over the world.

In some studies, “O” blood group among different blood groups was the most important common type of blood group as compared to AB blood group [2, 15]. The results of this type of study are also in according to the previous studies which were to be reported that blood group AB is known as the least of all the prevalent type of blood groups [2, 16-19]. Thus, in various groups with exceptional cases the gene for segregation of ABO blood group systems is followed in a special method for its distribution. In a Russian Federation study, “A” blood type of group was known to be the most important group [20]. In another study in Pakistan in Bannu Region, “B” blood group was reported as most prevalent group of the region. These of variations in prevalence of different blood groups are credited to sample sizes, ethnic groups, geographical and environment factors [21].

Antigen D is known as the strongest antigen among other antigen types from the blood group of Rh type and it causes problems in transfusion and antibody products. Thus, the classification of $Rh(D)^+$ and $Rh(D)^-$ depends on the presence of this antigen (D) [22]. This blood group will be considered as a single gene inheritance blood group because of the dominance of this antigen. Based on Rhesus (Rh) factor, the present study also confirmed that $Rh(D)^+$ has high rate frequency % (percentage) than $Rh(D)^-$ frequency percentage in Multan Division (3 studied districts) which was also observed in past studies on different types of ethnic groups [14, 23, 24]. In the African population the current and the past observations reported relatively very low cases of rhesus (Rh)⁻. However, we observe that our results are different in some studies among population of Bahawalpur Division of Punjab (Pakistan) where the incidences were considered

exclusively known as $Rh(D)^+$ [25]. The current study also could not find any high quantity of $Rh(D)^-$ same like the previous studies.

In any population having knowledge about ABO type and the Rh type of blood group systems amongst different groups of ethnic is necessary. Being of individuals in a population the types about the information obtained from the all findings are important for genetic counseling, medical diagnosis, genetic information as well as in general physiology. In this paper we report the frequencies of blood types for multiple types of blood group systems of a regional population in Multan Division (Punjab), Pakistan. In the ABO and the Rh(D) type of blood groups, no significant differences were considered to be found in frequency distribution as compared to that of the other regional data. In general, all of the three district groups are in the Hardy Weinberg Equilibrium but in district Lodhran group the equilibrium is stronger as compared to that of other two districts and the least was evaluated in the district Khanewal group. The data collected from this study would be most helpful for the expertise in such field of genetics population to explain the factors which are liable to be the observed in the distribution methods of such type of genetic constituent markers in such part of Multan Division, or even to whole Pakistan.

Conclusion

A valuable information regarding rhesus antigens, polymorphism about the blood group and genetic variability is highlighted by this study regarding three districts of Multan Division (Punjab), Pakistan. This information will provide basic facts and figures of this region to geneticists, practitioners and blood transfusion programmers.

Authors' contributions

Conceived and designed the experiments: M Zafar, Performed the experiments: M Zafar &

Asmatullah, Analyzed the data: MM Malik & S Masud, Contributed materials/ analysis/ tools: M Zafar, MS Noor, S Masud, MH Latif & Asmatullah, Wrote the paper: M Zafar.

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