Journal of Blood Medicine

Dovepress
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ORIGINAL RESEARCH

Phenotype, Allele and Genotype Frequency of ABO and Rhesus D Blood Groups of Blood Donors at the North Gondar District Blood Bank, Northwest Ethiopia

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Background: Knowledge of the ABO and RhD group distribution is essential for blood banks inventory and assuring quality blood transfusion services. The objective of this study was to determine the frequency of ABO and RhD phenotype, allele, and genotype among blood donors at North Gondar District Blood Bank from 2010 to 2012, Northwest Ethiopia.

Methods: The data of the current study were obtained from registration logbooks of blood donors registered. The ABO and RhD grouping was done by using commercially available monoclonal antibodies (anti-A, anti-B and anti-D) by slide methods. Results with no agglutination by anti-D antibody were confirmed using anti-human globulin test. Descriptive statistics were analyzed using SPSS version 20. The allele and genotype frequency of the donors was determined by Hardy–Weinberg equilibrium assumption. The difference between the observed and expected frequency was tested by online Chi-square calculator. P-value of <0.05 was considered statistically significant.

Results: Among 6471 blood donors, 82.1%, 94.1% and 55.4% were males, replacement donors and in the age group of 21–30 years, respectively. Blood group O (47.04%) and blood group AB (4.81%) were the dominant and least common, respectively. The distribution of the RhD negative blood group was 5.76%. The distribution of A, B and O alleles was 0.1714, 0.1433 and 0.6859, respectively. Moreover, the genotype frequency of AA, AO, BB, BO, AB and OO was 0.0294, 0.2350, 0.0205, 0.1966, 0.0491 and 0.4704, respectively. The genotype frequency of DD, Dd and dd was 0.5774, 0.3649 and 0.0576, respectively. The result showed that there was no statistically significant difference between observed and expected allele and genotype frequency (P-value >0.05).

Conclusion: Blood group O and AB were the most and least prevalent, respectively. The allele and genotype frequency of the population was fulfilled the Hardy–Weinberg equilibrium assumption. This finding might be useful for blood transfusion services.

Keywords: ABO blood group, blood donor, Ethiopia, RhD

Introduction

Blood is essential for transporting oxygen, nutrients, wastes and hormones in the body. The ABO blood group system is the most clinically important blood group system, which was discovered by Karl Landsteiner in 1900[1] and awarded the Nobel Prize in 1930. The fourth type of blood group was also discovered by Alfred Von Decastello and Adriano Sturli and named blood group AB, in 1902.[2] The rhesus

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Received: 14 November 2021
Accepted: 23 December 2021
Published: 6 January 2022

(Rh) blood group system is the second most significant blood group system. Depending on the presence of the D antigen on the red cell, the blood group system has two phenotypes; RhD positive and RhD negative. The ABO and RhD genes are found at chromosomes 9 and 1, respectively. Proteins and carbohydrates, bided to lipids or protein, are the blood group determining red blood cells antigens.[3]

Even there are about 100 blood group systems with 500 antigens, the ABO blood group systems are the most clinically important blood group systems in blood transfusion and organ transplantation services.[4,5] A and B antigens are highly antigenic and have naturally occurring antibodies in human plasma which are missing the corresponding antigen and can cause hemolysis in vivo. The Rh blood group system is the second most clinically significant blood group with regard to transfusion. In addition to this, they are also important in the context of genetic studies, identifying of medico-legal issues and tracing family history.[5]

The presence of ABO antigens in red blood cells is depending on glycosyltransferases, which add sugars to the antecedent substance. However, a specific sets of epitopes and the RhD protein make up the D antigen.[6]

The ABO blood groups are determined by using antisera (anti-A, anti-B and anti-AB) to detect the presence of A and B antigens. Additionally, known red cells antigens can be used to diagnose anti-A and anti-B in the plasma and called backward grouping. ABO and Rh phenotypes, allele and gene frequency differ broadly according to races and geographical borders.[3,7–9]

The frequency and distribution of ABO and Rh blood groups differ by ethnicity even in the same region. In Ethiopia, the frequencies of ABO and Rh blood groups have been investigated in some areas of the country. These studies showed that the frequency of the O blood group was the dominant followed by A, B and AB.[10–13] Concerning to Rh blood group, about 7.0 −19.37% of the donors were RhD negative[14] with the highest prevalence in Gambella (Ethiopia) (19.37%).[10]

Detection of the Rh system is imperative to avoid the erythroblastosis fetalis; which frequently occurred when an RhD negative mother carries an RhD positive fetus and during the second birth causes the death of the fetus. Knowing the frequency of ABO and RhD blood groups in a particular population is crucial for efficient management of blood bank inventor, even for local transfusion services.[9] Thus, the main objective of the current study was to determine the frequency and distribution of ABO and Rh blood groups among blood donors at the North Gondar district blood bank.

Methods and Materials

Study Area, Design and Population

This study was a retrospective study carried out at the University of Gondar hospital's distinct blood bank. The distinct blood bank is located at Gondar Town which was found 727 Km north of the capital city of Ethiopia, Addis Ababa. The blood groups of donors of either sex, donating during the period of three years (2010–2012) were studied. The blood bank provides transfusion services to approximately about five million people in North Gondar and neighboring districts. Annually, the blood bank collects about 2500 units of blood. The majority of the collected blood is used for emergency, surgical and gynecological cases.

The source population was all replacement and voluntary blood donors who donate at the University of Gondar Hospital's distinct blood bank and the study populations were donors who had donated between the year of 2010 and 2012. They are eligible for blood donation if donors are apparently healthy, none anemic (greater 12g/dl and 13g/dl hemoglobin for female and male, respectively) and aged between 18 and 65 years. The donors should be 45 kilograms and above for 350-millimeter unit donation and greater than 50 kilograms for 450-millimeter unit donation. Moreover, they should have normal vital signs (temperature <37^{co}, blood pressure; diastolic; 70–90 mmHg, systolic; 100–150 mmHg) and be free from any sexually transmitted infections including human immune virus, hepatitis B virus, hepatitis C virus and syphilis (Blood Donor Medical Assessment Guideline, National Blood Bank Service of Ethiopia, 2017).

Sample Size and Sampling Technique

All blood donors who donated blood at the North Gondar District Blood Bank from 2010 to 2012 were study populations. A total of 6471 blood donors were included. All the blood donors were selected by the censuses sampling technique.

Laboratory Tests

Blood grouping was determined by using commercially accessible blood grouping anti-sera; anti-A, anti-B and anti-D (ERYCLONE anti-A, anti-B and anti-D

monoclonal antibodies) by using the tile method. A drop of donor blood was placed on three places on a clean white tile. Then each antiserum was added and mixed with each blood drop with the help of an applicator stick. The presence of agglutination was indicating the presence of the corresponding Blood groups antigen. Hardy–Weinberg equilibrium assumption was used for the determination of the frequencies of alleles A, B, O, D and d, and expressed as a proportion.

Data Collection and Analysis

Demographic data of the donor like age, sex and occupation were obtained from the blood bank registration log book. The collected data were entered into Epi Info 3.5.1 and then transformed to SPSS Version 20 for analysis. The descriptive statistics were presented in the form of tables. The observed phenotype, allelic and genotypic frequency of the ABO and Rh blood group was compared by using chi-square test (calculated by online Chi-square calculator; available from https://www.icalcu.com/stat/chisqtest.html) considering the Hardy–Weinberg equilibrium assumption. According to Hardy–Weinberg assumption, the allelic and genotypic frequencies of the population will remain stable from generation to generation, provided that there is no mutation, no migration, and no natural selection in a large population with random mating. Therefore, if the locus of ABO with three alleles are, "O", "A" and "B" and then the frequency of the alleles "O", "A" and "B" will be designated by *r, p* and *q*, respectively and the frequencies of the O, A, B, and AB phenotypes are r^2, $(p^2 + 2pr)$, $(q^2 + 2qr)$, and 2pq, respectively.

So, the allele frequency of O, A and B can be estimated from the ABO phenotype by the following formula.

$$r = \sqrt{(frequancy\ of\ O\ phenotpe)}$$

$$p = \sqrt{(frequancy\ of\ A + O\ phenotpe)} - \mathrm{r}$$

$$q = \sqrt{(frequancy\ of\ B + O\ phenotpe)} - \mathrm{r}$$

The allele frequency obtained by this formula from the observed phenotype frequency is called observed or unadjusted allele frequency. It is clear that the sum of all allelic frequencies should be one. However, it is true if the observed frequencies of the phenotypes had no deviation from the expected values. However, due to some factors, the observed phenotype frequency may deviate from the expected value. Hence, the summation of the allele frequencies can also deviate from one. The deviation (d) can be calculated as follows; d= 1- (p+q+r); then the allelic frequencies should be corrected by:

$pc = p\left(1 + \frac{d}{2}\right), qc = q\left(1 + \frac{d}{2}\right)$, and $rc = 1 - (pc + qc)$ (corrected or adjusted allele frequency). The phenotype and genotype frequency, recalculated from the corrected allele frequency is called expected phenotype and genotype frequency, respectively and can be estimated from corrected allelic frequencies by the following formula.

Expected frequency of phenotype A: $p_c^2 + 2p_cr_c$ (AA genotype +2*AO genotype)

Expected frequency of phenotype B: $q_c^2 + 2q_cr_c$ (BB genotype +2*BO genotype)

Expected frequency of phenotype O: r_c^2 (OO genotype)

Expected frequency of phenotype AB: $2p_cq_c$ (2*AB genotype)

Concerning the Rh blood groups, there are only two phenotypes; RhD positive (DD + Dd) and RhD negative (dd). Therefore, if D and d alleles have p and q frequencies respectively, the frequency of RhD negative is equal to q^2 and the allele frequencies will be $\sqrt{q2}$ and p = 1- q. However, we cannot investigate the deviation, because we have no degree of freedom.[15]

Data Quality Assurance

The data quality was assured by following standard operation procedures, double entry. In addition, the quality of reagents used to determine the blood groups were checked by running the known blood sample.

Result

About 6471 blood donors have participated in the study. From these study participants 5311 (82.1%), 3586 (55.4%), 6089 (94.1%) and 1842 (28.5%) were male, 21–30 age group, replacement donor and students, respectively (Table 1).

ABO and Rh Phenotype, Allele, and Genotype Frequency

Out of the study participants 47.04%, 26.44%, 21.71% and 4.81% were had blood group O, A, B and AB phenotypes, respectively. The expected ABO phenotype distribution was 46.98%, 26.42%, 21.69% and 4.91% which was similar to the observed frequency (Chi-squared (3, N = 6471) = 0.16, P-value: 0.984). The current result also revealed that 94.24% of the donors were RhD positive. The observed and adjusted (corrected) allele distribution of A, B and O were 0.1714, 0.1433 and 0.6859; and 0.1713, 0.1433 and 0.6854,

Table 1 The Socio Demographic Characteristics of Blood Donors at University of Gondar Comprehensive Specialized Hospital Blood Bank (N = 4671)

Variables		Frequency	
		Number (n)	Percent (%)
Sex	Male	5311	82.1
	Female	1160	17.9
Age category	18–20	969	15.0
	21–30	3586	55.4
	31–40	1252	19.3
	41–50	508	7.9
	>50	156	2.4
Donor type	Voluntary	382	5.9
	Replacement	6089	94.1
Occupation	Student	1842	28.5
	Farmer	1728	26.7
	Government employed	947	14.6
	Private employed	296	4.6
	Self employed	1068	16.5
	Unemployed	590	9.1

respectively. The result showed that there was no significant difference between the observed and corrected allele frequency (Chi-squared (2, N = 6471) = 0; P-value: 1).

The observed ABO genotype frequency of the donors was 0.0294, 0.2350, 0.0205, 0.1966, 0.0491 and 0.4704 for AA, AO, BB, BO, AB and OO, respectively. On the other hand, the corrected ABO genotype frequency was 0.0293, 0.2348, 0.0205, 0.1964, 0.0491 and 0.4698 for AA, AO, BB, BO, AB and OO, respectively (had no significant difference from the observed genotype frequency; Chi-squared (2, N = 6471) = 0; P-value: 1). The Rh genotype frequency was 0.5774, 0.3649 and 0.0576 for DD, Dd and dd, respectively (Table 2).

Phenotype distribution of ABO and Rh blood group of donors also showed that there was no significant ABO and Rh phenotype distribution difference with age (chi-squared (28, N = 6471) = 27.86, P-value = 0.472). Moreover, the result showed that the ABO and Rh phenotype distribution had no statistical significance difference between male and female (chi-squared (7, N = 6471) = 7.649, P-value = 0.365) (Table 3).

Discussion

Knowing the distribution of ABO and RhD blood groups is imperative for blood bank inventory and transfusion services. It also helps to decide the way of mobilization of voluntary blood donors. In addition, understanding of ABO and RhD blood groups are valuable in studying population genetics, resettlement trend and settling certain medico-legal problems specifically disputed parentage and taking protective measures against blood group-associated diseases.[16]

The current result showed that most of the donors (89.7%) were from young age groups (<40 years old). The highest frequency of young donors in the current study might be due to over concentration on the selection of students' donors. The finding was similar to studies found in Madagascar[17] and Saudi Arabia[18] which reported that most of the donors were the youngest age groups. The least donors were found in >50 years old age group. This might be due that the elderly age groups might defer frequently than the young age groups. Studies revealed that deferral significantly increased as age increased due to abnormal blood pressure.[19,20]

In the current finding, most of the donors (82.1%) were males. This was comparable with other studies conducted in Madagascar[17] and India.[21–23] The reason might be the fact that males might be easily qualifying through the selection processes in donor recruitment. The other reason might be the difference in blood donation practice between males and females. Studies conducted in Ethiopia,[24,25] Nigeria[26] and Saudi Arabia[27] were supported that males had more blood donation practice than women. Indeed, other studies supported males and females had no significant difference in blood donation practice.[20,28,29] The other reason might be due to the high deferral rate in females. Studies showed that females were more defer than males[19,30,31] due to being more anemic compared to males.[19,20]

World Health Organization and other international and national organizations advised that blood donations must be voluntary and unpaid. This ensures the availability of a constant and safe blood supply.[32] However, this finding showed that the majority (94.1%) of the donors were replacement donors. This might be due to the poor blood donation practice in Ethiopia. Other researches done in the area demonstrated that blood donation practice was low.[25,33] The finding was similar to the studies conducted in Tanzania,[4] Egypt[34] and Saudi Arabia,[18] but, contradicted to a study conducted in India where the voluntary blood donors accounted for 87.75% of the donors.[35] The discrepancy might be attributed to difference in knowledge, attitude and practice of blood donation between the populations.

Table 2 Phenotype, Allele, and Genotype Frequency of ABO and Rh Among Blood Donors at University of Gondar Comprehensive Specialized Hospital Blood Bank (N = 6471)

ABO Phenotype	Observed Phenotype Frequency	Expected Phenotype Frequency	Chi Squared; P value	Unadjusted Allele Frequency	Adjusted Allele Frequency	Chi Squared; P value	Genotype Frequency			Chi Squared; P value
							Genotype	Unadjusted Frequency	Adjusted Frequency	
A	1711 (0.2644)	1710 (0.2642)	0.16; 0.984	0.1714	0.1713	0; 1	AA	0.0294	0.0293	0; 1
							AO	0.2350	0.2348	
B	1405 (0.2171)	1405 (0.2169)		0.1433	0.1433		BB	0.0205	0.0205	
							BO	0.1966	0.1964	
AB	311 (0.0481)	318 (0.0491)		–	–		AB	0.0491	0.0491	
O	3044 (0.4704)	3040 (0.4698)		0.6859	0.6854		OO	0.4704	0.4698	
Rh D										
RhD positive	6098 (0.9424)	6098 (0.9424)	–	0.7599	0.7599	–	DD	0.5774	0.5774	–
							Dd	0.3649	0.3649	
RhD negative	373 (0.0576)	373 (0.0576)		0.2401	0.2401		dd	0.0576	0.0576	

https://doi.org/10.2147/JBM.S346904 DovePress

Table 3 ABO Blood Group Distribution Regarding to Age and Sex (N = 6471)

Variables	Frequency	ABO and RhD Phenotype							
		A^{+ve} N (%)	A^{-ve} N (%)	B^{+ve} N (%)	B^{-ve} N (%)	AB^{+ve} N (%)	AB^{-ve} N (%)	O^{+ve} N (%)	O^{-ve} N (%)
Age									
18–20	969	259 (26.7)	11 (1.1)	210 (21.7)	11 (1.1)	33 (3.4)	1 (0.1)	419 (43.2)	25 (2.6)
21–30	3586	897 (25.1)	60 (1.7)	715 (19.9)	45 (1.2)	176 (4.9)	7 (0.1)	1586 (44.2)	100 (2.8)
31–40	1252	305 (24.4)	16 (1.3)	249 (19.9)	19 (1.5)	61 (4.8)	3 (0.2)	561(44.8)	38 (3.0)
41–50	508	110 (21.7)	5 (0.1)	111(21.9)	9 (1.8)	20 (3.9)	4 (0.8)	239 (47.0)	10 (2.0)
>50	156	46 (29.5)	2 (1.3)	34 (21.8)	2 (1.3)	6 (3.8)	0 (0.0)	61 (39.1)	5 (3.2)
Test statistics	Chi squared: 27. 86, degrees of freedom:28, P value: 0.472								
Sex									
Male	5312	1314 (24.7)	79 (1.5)	1074 (20.2)	68 (1.3)	247 (4.6)	15 (0.3)	2374 (44.7)	141 (2.7)
Female	1159	303 (26.1)	15 (1.3)	245 (21.1)	18 (1.6)	49 (4.2)	0 (0.0)	492 (42.5)	37 (3.2)
Test statistics	Chi squared: 7. 649, degrees of freedom:7, P value: 0.365								
Total	6471	1617 (25.0)	94 (1.5)	1319 (20.4)	86 (1.3)	296 (4.6)	15 (0.2)	2866 (44.3)	178 (2.8)

The genes accountable for the ABO blood groups have always taken a meticulous prototype for its circulation. In the current finding, the O blood group was the dominant ABO blood group with a frequency of 47.04%, followed by the A blood group (26.44%), B blood group (21.71%) and AB blood group (4.8%), which concluded that O>A>B>AB. Various other findings were showed that the O blood group was the most dominant blood group with the order of O>A>B>AB.[11,36–40] Of course, there was a difference in the proportion of the phenotype frequency. For example, the prevalence of the O blood group was 61.82% in Mexican Population[38] where as in the current study it was 47.04%. The variation might be due to race differences. In contrast to this finding, studies done in India[41] and Pakistan[42] revealed that the B blood group was the dominant phenotype, followed by O, A and AB blood group. On the other hand, studies done in Nepal[43] and Egypt[44] indicated that A blood group phenotype was the dominant blood group followed by O, B and AB. Moreover, studies in some European countries (Switzerland, Portugal and Greek) showed that A blood group was the dominant phenotype followed by O, B and AB.[45–47]

The current finding also showed that 94.24% and 5.76% of blood donors were RhD positive and RhD negative, respectively. This was similar to a study conducted in Kumaon,[16] Mexico[38] and Ethiopia.[11] However, this finding was different from the other findings done in Uganda,[48] Egypt,[44] Kenya,[40] Sudan[49] and Gambella (Ethiopia).[10] It showed that the RhD negative frequency was higher than Kenya (3.9% vs.5.76%) Sudan (2% vs 5.76% and Uganda (2% vs 5.76%) and it was lower than the t study done in Egypt (14.4% vs 5.76%) and Gambella (Ethiopia) (19.37% vs 5.76%).

In this study, the distribution of the allele A, B, and O were 0.1714, 0.1433 and 0.6859, respectively (allele frequency of O>A>B). This sequence was similar to the study done in Nigeria,[50] Cameroon[51] and Sudan.[52] However, it was different from the studies done in Guinea,[53] Nigeria[54,55] and Madagascar[17] where B allele frequency was higher than A allele frequency (O>B>A). On the other hand, the genotype frequency of AA, AO, BB, BO, AB and OO were 0.0294, 0.2350, 0.0205, 0.1966, 0.0491 and 0.4704, respectively. The finding revealed that the phenotypic distribution of the blood groups in the general study population groups were in agreement with Hardy–Weinberg equilibrium expectations (P-value > 0.05). The finding was supported by the other studies done in Ethiopia[11] and Cameroon.[51] However, a study done in Lagos (Nigeria) found significant

difference between the expected and the observed phenotypic frequencies of the blood groups, which violated Hardy–Weinberg law.[56]

Limitation of the Study

The method of blood group determination was done by using the tile slide method and this might miss the detection of weak antigens.

Conclusion

The current finding showed that the majority blood group was "O" and the least frequent was AB. Regarding Rh blood group, RhD positive takes the great share over RhD negative blood groups. The females' blood donation practice was very low and it is important to improve it by improving health status and the knowledge about blood donation. The current finding might provide knowledge about the genetic distribution and polymorphism of ABO and RhD blood groups among the blood donors at the University of Gondar hospital's distinct blood bank. This would be valuable to those who are interested in the genetic study, physicians and other stakeholders, particularly those who are involved in blood transfusion program activities.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author and can access on reasonable request.

Ethics Approval and Consent to Participate

This study was conducted per the Declaration of Helsinki. The study was conducted after obtaining ethical clearance from the Ethical review board of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Science, University of Gondar. Permission was also obtained from the University of Gondar Hospital Laboratory head before the data collection. The data was accessed and complied with relevant data protection and privacy regulations by using anonymous. However, since the study was a retrospective review of blood donors' records, informed consent from the study participants was not sought.

Acknowledgment

The authors like to acknowledge the School of Biomedical and Laboratory Sciences, College of Medicine and Health Science, University of Gondar and University of Gondar Hospital Laboratory head.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

There was no funding source for this study.

Disclosure

The authors declare that there have no conflicts of interest.

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