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Frequency and Distribution of ABO and Rh (D) Blood Group Phenotype, Allele Frequencies and Genotypes Among Blood Donors in South Wollo, Northeast, Ethiopia

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ABSTRACT

Background: For safe blood transfusion services, understanding ABO blood group and Rhesus factor distribution at local levels is very essential.

Objective: To determine the frequency of phenotype, allele and genotypes of ABO and Rhesus (D) blood group in South Wollo, Ethiopia.

Materials and Methods: A cross-sectional document review was done using data from September 2019 to March 2023. The Chi-square test was used to assess the variations in the distribution of blood groups based on sex and locations, as well as to compare the observed and expected frequencies with data from various sites in Ethiopia. The frequencies of alleles and genotypes were calculated under Hardy-Weinberg assumption.

Result: 16,318 participants with median age of 26 years were included in the study, 11,924 males. The most common blood groups were O (40.4%) and Rh (D) positive (90.7%). Allele of I^O and I^OI^O were the predominant allelic and genotypic frequency respectively. In the case of Rh the dominant allele D was the most common. ABO and Rh frequency differ significantly across different sites. However, there is no difference in the ABO and Rh blood groups between the current study and other sites of Ethiopia as well as between observed and expected blood groups frequencies.

Conclusion: the most and the least blood groups were O and AB respectively. The distribution of ABO and Rh blood groups varies among different locations. No significance difference of ABO and Rh blood groups between present study and other areas of Ethiopia.

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Abbreviations

ISBT: International Society of Blood Transfusion; Rh: Rhesus factor.

Materials and Methods: All methods were carried out in accordance with relevant guidelines and regulations

Data Availability: All the data used for the analysis of this manuscript are available in the corresponding author

Ethical Considerations: We obtained ethical clearance from college of medicine and health science ethical clearance committee and permission letter from Dessie blood bank management bodies. Written informed consent was obtained from each study participant.

Consent for Publication: Consent for publication of this work was found from Dessie Blood Bank.

Introduction

Humans have 4 to 6 liter of blood containing erythrocytes, leukocytes and thrombocyte; blood involved in respiration, nutrition, immune defense, thermoregulation, and acid base regulation, and waste elimination. The categorization of blood into types relies on whether specific antigenic substances or blood groups are present or absent on the surface of red blood cells (RBCs). Each blood group system is represented by definite antigens found on the surface of red blood cells. Currently the International Society of Blood Transfusion (ISBT) has recognized, greater than about 700 human blood group antigens organized into 42 systems. The popular blood grouping systems are ABO blood group system and Rhesus (Rh) blood group system despite the long list of several other blood groups discovered so far [1-3].

Karl Landsteiner discovered ABO and Rh blood grouping systems in homosapians and recommended the letters O, A, B, and AB to express blood groups. ABO gene has mainly three types of alleles: i expresses no antigen hence type O, IA expresses type A antigen, and IB expresses type B antigen where I is a designation for iso-agglutinogen. Rh is the second major blood group system which comprises more than 50 blood group antigens, of which 'D' is most immunogenic and Rh-D typing is the part of routine blood grouping procedures [4,5].

The ABO and Rh blood group antigens are inherited traits that are valuable for studies in population genetics and migration patterns, organ transplantation, pregnancy, and addressing certain legal matters, especially those concerning disputed paternity. More critically, the Rh system plays a vital role in preventing the risk of erythroblastosis fetalis during pregnancy [6]. The ABO blood system is widely being studied in relation to susceptibility to infectious as well as noninfectious diseases in different human populations. Research on the ABO blood system is prevalent in exploring its relationship with susceptibility to both infectious and non-infectious diseases across various human populations. This blood group system has been linked to conditions such as ace, epithelial ovarian, gastric cancer, and other non-communicable diseases like ischaemic heart disease and diabetes mellitus. Additionally, the relationship between malaria and the ABO blood group is currently receiving increased focus among infectious diseases [7-12].

Information regarding the distribution of ABO and Rh blood groups can greatly assist in the management of blood banks and ensure safe transfusions. Blood groups distribution of any region can be influenced by race, ethnicity, geographical conditions, genetic drift, and migration frequency of population. In addition the ABO-Rh blood group frequencies may change temporally in a single population [13]. Environment factors and natural selection for survival of population in that region also affects the blood group distribution.

Understanding the distribution of ABO and Rh blood types at both local and regional levels is crucial for the efficient operation of blood banks and the provision of safe blood transfusion services, as well as for recognizing disease associations related to blood groups and preparation of donor data for organ transplantation. In addition, the need for estimates of blood group and gene's frequency studies provides very valuable information on the genetic similarity of different populations and to some extent on their ancestral genetic linkage, forensic medicine and anthropology.

In Ethiopia, the distribution of the ABO-Rh blood group system is little explored. There has been no known data of the distribution pattern and frequency of ABO and Rh blood groups from South Wollo Zone Northeast, Ethiopia. Thus, the objective of this study was to determine the distribution of phenotype, allele and genotype frequencies of ABO and Rh (D) blood groups in this region.

Materials and Methods

Study Area

The study was conducted from September 2019 to March 2023 in South Wollo Zone Northeast, Ethiopia. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), South Wollo has a total population of 2,518,862, of whom 1,248,698 are men and 1,270,164 women; the Zone has one earliest blood bank, called Dessie blood bank and it provides blood and blood components for all surrounding health institutions.

Study Design and Period

A cross-sectional document review study was done using the data from September 2019 to March 2023 by reviewing blood donor registration computer at Dessie blood bank

Date Collection

A checklist from the computer or software was used to review and retrospectively record data about sex, age, place of residence, and ABO and Rh blood group phenotypes

Inclusion and Exclusion Criteria

Recruitment of blood donors: For the donors to be eligible, they had to be between the ages of 18 and 60, weigh more than 45 kg, have normal blood pressure, and have hemoglobin levels of at least 120 g/L for women and 130 g/L for men. A donor whose documents was inconsistent, incomplete, or lost, was excluded from the study. The donors those donated their blood repetitively and recorded more than once were considered only once for the study.

Laboratory Tests

Blood samples were collected during donation using a plain tube. Reverse blood typing was performed utilizing established 5% A and B cell suspensions prepared in the laboratory, while forward ABO blood phenotyping was conducted via the slide technique using known commercial antisera anti-A and anti-B. The Coombs test was employed to detect weak D antigens, and the test tube method was used to determine the Rh group with anti-D reagents. The Rh-D and ABO phenotypes of the blood donors were recorded in the computerized blood donor data management system.

Date Quality Control

The quality of the data was guaranteed by adhering to standard operating procedures, performing double data entry, and conducting checks for completeness and consistency. Furthermore, the quality of the reagents utilized for blood group determination was verified by testing with known blood samples.

Data Processing, Analyses and Interpretation

All data was entered and analyzed using SPSS version 25 and taking care for completeness, consistency, and coding. Prior to conducting any statistical analysis, descriptive statistics such as frequencies and percentages for the variables were calculated to summerize the data. The Chi-square test was employed to examine the differences in observed phenotype frequency distributions of the ABO and Rh blood groups. Also we employed a chi-square test to test the presence of ABO and Rh blood group distribution difference by sex, blood donation sites and difference between present study and other study sites in Ethiopia. In all cases, p-value ≤ 0.05 was taken as a statistically significant association. To determine which cells in the contingency table are substantially different, the Bonferroni post hoc test was used. Finally, the finding was presented with texts and tables.

The allelic and genotypic rates of the ABO blood groups were calculated from phenotypic frequencies using a modified Hardy-Weinberg equation with more than two alleles. Rule of probability theory can be applied to any quantity of alleles that are passed down to a diploid zygote at a time. Three alleles (I^A, I^B, and I^O) were calculated for this research, with frequencies of p, q, and r, respectively. The distributions of blood groups among blood donors were first expressed in this research as percentages and frequencies. Observed percentage frequency =observed number/ total number times×100

Allelic frequency determination of ABO blood groups frequency of the three ABO blood group alleles (p, q and r) was determined by using observed percentage frequency as follows from:

$r = (O)^{1/2}$, $p = 1 – (B+O)^{1/2}$ and $q= 1- (A+O)^{1/2}$

Frequencies of the two Rh (D) blood group alleles I^D and I^d (p and q) respectively, were determined as follows: $q = (Rh-)^{1/2}$ and $P = 1 – q$

Using common population genetics formulas, the gene frequency was determined when taking into account two alleles at the same locus for the Rh system and three alleles at the same locus for

ABO. By using trinomial expansion, the genotype rates at equilibrium are calculated as follows: $(p+q+r)^2$; p^2 (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO) = 1.
Where; P^2 is the frequency of genotype $I^A I^A$, q^2 is the frequency of genotype $I^B I^B$, 2pq is frequency of genotype $I^A I^B$, 2pr is frequency of genotype $I^A I^O$, 2qr is the frequency of genotype $I^B I^O$ and r^2 is the frequency of genotype $I^O I^O$
Formula for the genotypic frequencies of Rh (D) blood groups are calculated as follows:
Genotype DD = p^2 Genotype Dd = 2pq and Genotype dd = q^2
Expected phenotypic frequencies (Ef) were calculated as: Ef = Genotypic frequency X number of total sample: **i.e**. for A blood group Ef = frequency of (AA + AO) X number of total sample

Result

Socio-Demographic Characteristics of the Study Participant

A total of 17,806 donors donated blood in the study period. From which 1488 blood donors were donated blood repetitively and recorded more than once. Finally a total of 16,318 participants were included in the study, 11,924 males and 4394 females. Age ranges from 18-64 years with median age of 26 years Table 1. All participants donate blood voluntarily and without pay. No significant difference of ABO and Rh (D) between gender and age.

Table 1: Age and Sex Distribution of Voluntary Non-Remunerated Blood Donors in South Wollo

	Male					Female				
	18-25	26-35	36-50	51-65	Total	16-25	26-35	36-50	51-65	Total
Frequency	5795	3835	2279	343	12252	3134	635	264	33	4066
%	47.3	31.3	18.6	2.8	100	77.1	15.6	6.5	0.8	

Frequency of ABO and Rh blood Grouping

In this study the most common blood group was blood group O (40.4%) followed by A (30%), B (24.3%) and AB (5.3%). Regarding Rh blood group Rh (D) positive was the most frequent (90.7%), while the Rh (D) negative blood group was (9.3%) Table 2. Considering ABO and Rh D) blood group overall blood group O positive was the most frequent with constituents 36.5% and AB positive was the least (5%). Similarly, ABO and Rh (D) negative altogether O negative was the most frequent (3.9%), while AB negative was the least frequent blood group (0.5%) Table 3. The observed and expected frequencies of ABO and Rh (D) blood groups were not significantly different Table 4.

Table 2: ABO and Rh (D) Blood Groups and Sex distribution of Voluntary Non-Remunerated Blood Donors in South Wollo

	A (%)	B (%)	O (%)	AB (%)	Rh +ve (%)	Rh-ve (%)
Female	1305	979	1821	289	4004	390
Male	3592	2954	4770	608	10792	1132
Total(%)	4897(30)	3933(24.3)	6591(40.4)	897(5.3)	14796(90.7)	1522(9.3)

Table 3: ABO with Rh (D) Blood Group and Sex Distribution of Voluntary Non-Remunerated Blood Donors in South Wollo

Gender	A+	A-	B+	B-	O+	O-	AB+	AB-	Total
Female	1188	117	890	89	1673	148	253	36	4394
Male	3281	311	2672	282	4284	486	555	53	11924
Total (%)	4469 (27.4)	428 (2.6)	3562 (21.8)	371 (2.3)	5957 (36.5)	634 (3.9)	808 (5)	89 (0.5)	16318 (100)

Table 4: Observed and Expected Numbers and Frequencies of ABO and Rh (D) Blood Groups Among Blood Donors in South Wollo

ABO Blood system					Rh(D) blood system				
ABO Blood group	Observed number	Observed frequency	Expected number	Expected frequency	Rh(D) blood group	Observed number	Observed Frequency	Expected number	Expected frequency
O	6591	0.404	6590.84	0.4039	Rh +ve	14796	0.907	14797.16	0.9068
A	4897	0.30	4676.74	0.2866	Rh-Ve	1522	0.093	1515.94	0.0929
B	3933	0.243	4436.86	0.2719					
AB	897	0.093	1026.40	0.0629					
Total	16318	1			total	16318	1		
X2	X2= 12,	df = 9	P= 0.213		X2 =2	Df= 1	p = 0.157		

Distribution of Allelic and Genotypic Frequency of ABO and Rh (D) Blood Groups in South Wollo
Allele frequency of IO was the predominant allele which is 0.6356 followed by IA =0.1956 and IB =0.161. In the case of Rh blood group allele the dominant allele D and the recessive allele d were 0.695 and 0.3049 respectively. The genotypic frequency of IOIO was the most frequent 0.4039 followed by IAIO=0.2486, IBIO=0.2046, IAIB=0.0629, IAIA=0.038 and IBIB=0.0259table 5. The genotypic frequency of IOIO was the most frequent 0.4039 followed by IAIO (0.2486), IBIO (0.2046), IAIB (0.0629), IAIA (0.038)

and IBIB (0.0259). In the case of Rh blood group allele the dominant allele D was the most common (0.4830) followed by Dd (0.4238) and dd (0.0929) Table 5. There is no difference in the phenotype frequency of ABO and Rh blood groups between the current study and other sites of Ethiopia Table 6. Similarly, the allelic frequencies also have no difference between the current study and other sites of Ethiopia Table 7.

Table 5: Genotypic and Allelic Frequencies of Combined ABO and Rh Blood Groups Among Blood Donors

Blood group	Gene (Allele)	Allelic Frequency	Genotype	Genotypic Frequency	Phenotype	Phenotypic Frequency
ABO	O	0.6356	OO	0.4039	O	0.404
	A	0.1956	AA	0.038	A	0.3
			AO	0.2486	A	
	B	0.161	BB	0.0259	B	0.243
			BO	0.2046	B	
			AB	0.0629	AB	0.053
Rhesus	D	0.695	DD	0.4830	Rh+ve	0.907
			Dd	0.4238	Rh+ve	
	d	0.3049	dd	0.0929	Rh- vE	0.093

Table 6: Comparison of ABO and Rh (D) Phenotyping Frequencies Between South Wollo and other Populations in Different Regions of Ethiopia

Study	ABO type (%)				Rh (D) type (%)		Reference
	A	B	O	AB	Rh+	Rh-	
Present	30	24.3	40.4	5.3	90.7)	9.3	Present study
Silte Zone	28.11	23.35	43.08	5.44	92.06	7.94	(1)
Gambella	34.96	20.48	41.20	3.34	80.62	19.37	(14)
Woldia	28.20	25.40	21.67	24.70	60.13	39.86	(15)
Arba Minch	32.7	20.9	42.1	4.3	92.8	7.2	(16)
Amhara	28.7	22.2	41.6	7.7	92.5	7.5	(17)
Bahr Dar	29.8	23.2	41.5	5.5	91.5	8.5	(18)
Debre Tabor	29.48	24.06	39.6	6.7	92.77	7.23	(19)
Jimma	31.9	21.5	43.1	3.5	92.8	7.2	(20)
Nekemte	29.5	20.2	45.6	4.7	93	7	(21)
	χ^2= 12	P= 0.213			χ^2= 2	P= 0.157	

Table 7: Comparison of ABO and Rh (D) Allele Frequencies Between South Wollo and Other Populations in Different Regions of Ethiopia

Study	Allele frequencies						Reference
	A	B	O	AB	D	d	
Present	0.3	0.243	0.404	0.053	0.907	0.093	Present study
Silte Zone	0.19	0.15	0.65	NA	0.72	0.28	(1)
Gambella	0.2305	0.1277	0.6418	NA	NA	NA	(14)
Woldia	0.314	0.2939	0.4655	NA	0.3687	0.6313	(15)
Arba Minch	NA	NA	NA	NA	NA	NA	NA
Amhara	NA	NA	NA	NA	NA	NA	NA
Bahr Dar	NA	NA	NA	NA	NA	NA	NA
Debre Tabor	NA	NA	NA	NA	NA	NA	NA
Jimma	NA	NA	NA	NA	NA	NA	NA
	P> 0.157				P> 0.157		

NA; Not applicable

Table 8: Distribution of ABO and Rh Blood Groups with Blood Donation Site

Site of donation	ABO				Rh	
	A	B	O	AB	Rh +ve	Rh -ve
Dessie	1999 (30.2%)	1579(23.8%)	2666(40.2%)	387(5.8%)	6116(92.2%)*	515 (7.8%)*
Kombolcha	453 (27.6%)	397(24.2%)	693(42.3)	97 (5.9%)	1476(90%)	164 (10%)
Sayint	204 (34.9%)	128(21.9%)	213(36.4%)	40(6.8%)	511(87.3%)*	74 (12.7%)*
Akasta	201 (27%)	194 (26.1%)	319 (42.9%)	39 (5%)	670(90.2%)	73 (9.8%)
Albuko	49 (29.3%)	40 (24%)	66 39.5%)	12 (7.2%)	145 (86.8%)	22 (13.2%)
Argoba	24 (34.8%)	18 (26.1%)	20 (29%)	7 (10.1%)	62 (89.9%)	7 (10.1%)
Bistima	95 (32.7%)	65 (22.3%)	106 (36.4%)	25 (8.6%)	249 (85.6%)	42 (14.4%)
Delanta	139 (30.8%)	103 (22.9%)	185 (40.9%)	25 (5.5%)	417 (92.3%)	35 (7.7%)
m/selam	397 (27.3%)	373(25.6%)	646 (44.4%)	39(2.7%)	1307(89.8%)	148 (10.2%)
kelela	114 (28.4%)	113(28.2%)	157 (39.2%)	17 (4.2%)	349 (87%)	52 (13%)
Mekdela	119 (31%)	99 (25.8%)	142 (37%)	24 (6.2%)	346 90.1%)	38 (9.9%)
Tenta	131(32.8%)	103 (25.7%)	144 (36%)	22 (5.5%)	365 (91.2%)	35 (8.8%)
Wuchale	102 (32.9%)	71 (22.9%)	120 (38.7%)	17 (5.5%)	272 (87.7%)	38 (12.3%)
Wegidi	151 (33%)	101 (22.1%)	177 (38.7%)	28 (6.1%)	410 (89.7%)	47 (10.3%)
Werielu	364 (31.8%)	270 (23.6%)	451(39.4%)	59 (5.2%)	1036(90.6%)	108 (9.4%)
Jamma	170 (31.4%)	121 (22.4%)	216(39.9%)	34 (6.3%)	500 (92.4%)	41 (7.6%)
Haik	124 (28.8%)	101 (23.4%)	198 (45.9%)	8 (1.9%)*	390 (90.5%)	41 (9.5%)
Kutaber	61 (29.5%)	57 (27.5%)	72 (34.8%)	17 (8.2%)	165 (79.7%)*	42 (20.3%)

*Donation sites with, significantly, higher or lower proportions of B, AB, O, and Rh-positive blood group (adjusted P=0.0007, and d <0.0014 respectively).

Discussion

A retrospective study was conducted on 16,318 South Wollo Zone to determine ABO and Rh (D) phenotype, allele frequencies and estimated genotypes. The blood group typing is very useful in the genetic population, so the study of prevalence and frequencies should be carried out in every central and regional blood bank.

In this research, there was a greater participation in blood donation among males compared to females which accounted for 73.1%, while 26.9% were females in line with our finding studies done in Ethiopia [14-21]. This may be attributed to female donors being more likely to differ from male donors for medical reasons, such as having low hemoglobin levels, lower body weight, being pregnant, breastfeeding, or fearing needle pricks. Among all the blood donors in our study, the largest group consisted of younger individuals (ages 18–25), which aligns with findings from studies. This trend could be explained by the fact that this demographic is active, easily persuaded, and influenced by their peers. However, a study conducted in North Coa showed majority donors belonged to the age group 31-40 years and a study conducted in Yemen majority of donors were age b/n 25-31. Also, the majority 69.6% (n=45 595) were aged ≥35 years in a study conducted in Kilimanjaro, Tanzania [22-24]. The difference may be due to differences in knowledge and attitude towards blood donation and blood donation customs among the various populations.

The overall ABO distribution pattern in South Wollo Zone showed group O is the highest (40.4%), the second-commonest blood group is A (30%), the third commonest is B (24.3%), and AB (5.3%) is the least blood group which is similar to those reported in different sites of Ethiopia. And other areas in the world like Mogadishu-Somalia, Republic of Congo, Abakaliki, Iranian. This ABO distribution is different from those in Egypt and Nigist Eleni Mohammed Hospital, Ethiopia where type A is the commonest, and B was the predominant blood group type in Delhi, India, South Gujarat India and AFIT Rawalpindi. Similarly a studies conducted in Ponciry, Saudi Arabia and Chhattisgarh Teaching Hospital indicated that group B was the second most prevalent blood type 33.5% next to group O. The variation in the frequency distribution of ABO blood groups may be a result of genetic differences among the study participants [25-37].

Rh (D) positive and negative phenotype frequencies vary between populations. Present study recorded Rh (D) positive frequency of 90.7% and Rh (D) negative frequency of 9.3%. This is similar to some countries in. However, the frequency Rh (D) negative in this study is less when compared to where its frequency is as high as 39.86% and 19.37% respectively. Considering ABO and Rh blood group altogether, blood group O positive (37.9%) was the most predominant blood group whereas AB negative (0.4%) was the rarest; similarly, a study in Ethiopia. The blood bank needs to recognize that only 3.9% of all donors belong to the "O" Rh-D negative group, even though this blood type is considered the universal cell donor, potentially benefitting numerous patients in emergency transfusion situations.

Based on the adjusted Hardy-Weinberg Law of equilibrium, the determined allelic frequencies of the ABO blood types among the blood donors in this research were the frequencies of the alleles IO is the commonest (0.6356), IA is the next (0.1956) and IB, is the least common (0.1610). Rh (D) ID is the most common (0.6950) while, Id is the least one (0.3049). Previous studies among various parts of the world population have documented similar patterns of allelic frequencies. However, studies conducted in Teaching Hospital of Chhattisgarh, and South India, Delhi, India [38]. IB was the second most frequency alles next to Io and the allele

frequencies follow an order of $I^O > I^B > I^A$ and Rh- $I^D > I^d$.

Regarding the genotype frequencies of ABO blood groups I^OI^O the most prevalent which was 0.4039 and the least genotypic frequency was I^AI^B 0.0629, which is similar with studies conducted in. In contrast with study conducted in Delhi, India, Genotype frequencies follow an order of BO > OO > AO > AB > BB > AA.

The distribution of ABO and Rh blood groups differs across various blood donation locations, allowing local blood banks to utilize this information to modify their blood inventory based on blood type. However, when we compared present study of the overall ABO and Rh blood group frequencies and with those from studies conducted in other areas of Ethiopia, no statistical significance existed between them.

The actual and anticipated frequencies of ABO and Rh (D) blood types showed no significant differences. This result indicated that the phenotypic distribution of blood types in the general study populations aligned with the predictions of Hardy–Weinberg equilibrium.

Conclusion

In this study, blood group O was the most common followed by A, B, AB. Most of the blood donors' blood groups were Rh-D positive. Allele frequency of IO and ID were the predominant alleles. Similarly, the genotypic frequency of IOIO was the most frequent. There were no association between ABO and Rh- D with gender and age. The population in this region is in Hardy-Weinberg proportion, since, the observed and expected frequencies of ABO and Rh (D) blood groups were not significantly different. In this study the phenotyping frequency of those blood groups were not significantly different from other studies in different parts of Ethiopia. This may indicate in Ethiopia blood group O and the Rh- positive were the dominant blood groups. However, in this region showed that ABO and Rh blood group distribution varies among different blood donation sites so that the local blood bank will make use of this finding to adjust bloodstock by blood type.

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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, agree to be accountable for all aspects of the work.

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