



Distribution of ABO, Rhesus D and Subgroups of ABO among Blood Donors in Sokoto, North Western Nigeria

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Authors' contributions

This study was carried out in collaboration among all authors. Authors ABI and HA designed the study, performed the statistical analysis. Authors OE, PFU, AY and HAB wrote the protocol and first draft of the manuscript. Authors HMA and FUU managed the analyses of the study. Authors DI, UA and MUK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

ABO, Rhesus D and subgroups of ABO are highly immunogenic and are the common cause of antibody production in mismatched blood transfusions, haemolytic transfusion reaction and maternal alloimmunization. The aim of this study was to determine the occurrence of ABO, Rh D and subgroups of ABO among blood donors attending Specialist Hospital Sokoto, Nigeria. ABO, Rhesus D and subgroups of ABO antigen status of 176 blood donors with mean age of 30.44 ± 8.210 years

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attending Specialist Hospital Sokoto were determined using tile method for ABO and Rh D and conventional tube method for anti- A₁, anti- H reagents for ABO subgroups respectively. Among the 176 subjects tested, blood group O⁺ was the most frequent group with 93 (52.8%), 39 (22.2%) were blood group B⁺, 37(21.0%) were blood group A⁺, 5 (2.8%) were blood group AB⁺, 2 (1.1%) were blood group O⁻. No data was obtained for A⁻, B⁻ and AB⁻ blood groups. Out of 37 A blood groups obtained, 31 (83.8%) had A₁ antigens and 6 (16.2%) had A₂ antigens. Out of the 5 AB blood groups, all had A₁B antigens. The study also shows that there was statistically significant difference between blood group A and ethnic groups (Hausa, Fulani and Yoruba) (p<0.05). Blood group O was found to be the most frequent followed by B, A and AB except among Hausa which revealed a pattern of O> A> B> AB. ABO, subgroups shows majority had A₁ followed by A₂ and A₁B respectively.

Keywords: Blood donors; specialist hospital; Sokoto; ABO; Rh D and ABO subgroups.

1. INTRODUCTION

ABO blood group was the first blood grouping system discovered by Landsteiner [1]. The Discovery of ABO blood groups in 1901 marked the beginning of safe transfusion practice. Till today, it is the most important blood group system in transfusion medicine [2]. There are four main blood groups enlisted in this system namely A, B, AB and O [3]. Subtypes of A antigen have been defined, based on which A and AB blood groups have been classified into two main subgroups each. Approximately, 20% of individuals having A₂ antigen in blood belong to A₂ and thus, forming either A₂ or A₂B subgroups while rest belong to A₁, so as to form either A₁ or A₁B subgroups [4]. Sujata et al. [5] suggest A₁ and A₂ are distinguished by the reactivity of lectin i.e., anti-A₁ which occurs as a cold agglutinin and exclusively agglutinates A₁ cells. About 0.4% A₂ and 25% of A₂B subgroups possess anti-A₁. These antibodies become clinically significant if they react at 37°C destroying A₁ cells.

The Rh blood group system is the second most important blood group system, after the ABO blood group system. The Rh blood group system consists of 49 defined blood group antigens, among which the five antigens D, C, c, E, and e are the most important. There is no d ("lower case") antigen. Rh(D) status of an individual is normally described with a positive or negative suffix after the ABO type (e.g., someone who is A Positive has the A antigen and the Rh(D) antigen, whereas someone who is A Negative lacks the Rh (D) antigen) [6]. The terms Rh factor, Rh positive, and Rh negative refer to the Rh (D) antigen only. Antibodies to Rh antigens can be involved in hemolytic transfusion reactions and antibodies to the Rh (D) and Rh(c) antigens confer significant risk of haemolytic

disease of the fetus and newborn. Rhesus antigens are highly immunogenic. Out of 49 Rh antigens identified till now, D antigen is most significant [6]. Rhesus D negative individuals produce anti-D if they encounter the D antigen through transfusion or pregnancy and causes hemolytic transfusion reaction, or hemolytic disease of fetus and newborn. For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be [6].

Sokoto is the capital city of Sokoto State, Nigeria. The State is located between longitude 11°30', 13°50' East and latitude 4° to 6°0' North. It is bordered to the North by Niger Republic, Zamfara State to the East and Kebbi State to the South-West [7]. The State falls within the Savannah vegetation zone. Rainfall starts late and ends early with mean annual rainfall ranging from 500 to 1,300 mm. The State has three peculiar seasons; the cold dry (November-February), hot dry (March-July) and wet. The wet season begins in most part of the State in May and lasts up to September. The State is made up of Hausa and Fulani as the majority and a minority of Zabarmawa and Tuareg and other non- indigenous settlers. The two major languages in the State are Hausa and Fulfulde (spoken among the Fulani). The main occupation of the people is grain production and animal husbandry. Majority of the indigenous people practice agriculture. Crops produced include; commercial crops like millet, sorghum, beans, rice and maize. Other occupations commonly practiced are dying, blacksmithing, weaving, carving, trading, and cobbling. Sokoto ranks second in livestock production in Nigeria. Modern Sokoto city is a major commercial centre in leather crafts and agricultural products. Occupation of city inhabitants also include trading, commerce, with a reasonable proportion

of the population working in private and public sectors. The vast Fadama land of the Sokoto-Rima River system dissects the plain and provides rich alluvial soil and extensive grassland fit for a variety of crop cultivation, hence farming and livestock rearing are the principal activities in the State [8]. Other commercial activities are cement and leather production. The leather industrial sites may serve as breeding sites for mosquitoes as most of the wells are uncovered and the water disposal system is poor. Based on 2006 population census, Sokoto State had a population of 3,696,999 with a projected population of 5,399,467.12 in 2020 based on the population annual growth rate of 3% [9].

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross-sectional study designed on 176 blood donors attending Haematology unit of Specialist Hospital Sokoto for blood donation.

2.2 Study Area

This study was conducted in Specialist Hospital Sokoto, Department of laboratory services, Haematology unit.

2.3 Study Population

A total of one hundred and seventy-six (176) blood donors were enrolled into the study. All the subjects were recruited at Specialist Hospital Sokoto and their ABO, Rhesus D and subgroups of ABO were evaluated.

2.4 Inclusion Criteria

Blood donors that are between eighteen to sixty (18-60) years of age who are willing to give informed consent were included in the study.

2.5 Exclusion Criteria

Blood donors who refuse to give informed consent were excluded from this study.

2.6 Study Instruments

2.6.1 Questionnaire

A semi structured interviewer questionnaire was administered to all consenting participants to obtain donor information such as bio-data, socio-demographic characteristics and medical history.

2.7 Sample Size Determination

The sample size was calculated using the standard formula for calculating minimum sample size [10].

$$n = \frac{Z^2Pq}{d^2}$$

Where;

n =Minimum sample size

Z=Standard normal deviation at 95% level of confidence = 1.96

P=20.78% [11].

q=Compliment of p i.e. 1-p = 1-0.10 = 0.7922

d=Tolerance margin of error = 94% i.e. (100-94%) = 6% = 0.06

Therefore

$$n = \frac{(1.96)^2 \times 0.2078 \times 0.7922}{(0.06)^2} = \frac{0.6324}{0.0036} = 175.67 \approx 176$$

The calculated sample size is 176.

2.8 Blood Sample Collection and Processing

From each selected subject, a total of three millilitres (3 ml) of venous blood sample was drawn aseptically into an K₂EDTA anticoagulated container (2 mg/ml of blood) and labelled by assigning an identification number to each sample collected.

2.9 Laboratory Methods

2.9.1 ABO and Rhesus D grouping method

Principle of ABO and Rhesus D blood group:

The ABO and Rh D blood grouping is based on agglutination reaction. When red blood cells carrying one or both the antigens are exposed to the corresponding antibodies they interact with each other to form visible agglutination or clumping. The ABO blood group antigens are O-linked glycoproteins in which the terminal sugar residues exposed at the cell surface of the red blood cells determine whether the antigen is A or B. Blood group A subjects have A antigen on RBCs and anti-B antibodies in serum. Likewise, blood group B subjects have B antigens on RBCs and anti-A antibodies in serum. Blood group AB subjects have both A and B antigens on RBCs and neither anti-A nor anti-B antibodies in serum. Whereas, blood group O subjects have neither A antigens nor B antigens, but has both anti-A and anti-B antibodies in serum. The Rh

antigens are transmembrane proteins in which the loops exposed on the surface of red blood cells interact with the corresponding antibodies [12].

Procedure of ABO and Rhesus D grouping (Tile Technique): The method used for the determination of ABO and Rh D blood groups is the Ag-Ab agglutination test using the tile technique. This involved mixing of one drop of donor's whole blood with one drop each of commercially prepared anti-A, anti-B, anti-AB, and anti-D on a plastic tile. Using a glass rod, the cells and sera is mixed, and the tile rocked gently for a minute. Agglutinations are read after 2 mins macroscopically [13].

2.9.2 ABO subgrouping test principles and procedures

Blood groups of A and AB obtained in ABO grouping above was further sub grouped into A₁, A₂, A₁B and A₂B. Anti-A₁ and Anti-H lectins were used and the principle and procedure were as follows:

ABO subgrouping test principle: The reagent anti-A₁ Lectin and anti-H lectin causes agglutination of red cells, that carry the A₁ antigen or H antigen, after centrifugation. No agglutination generally indicates the absence of the A₁ antigen or H antigen.

ABO subgrouping test procedure (Tube technique): A 2-3% suspension of red cells was prepared in isotonic saline and A labeled test tube was placed: 1 volume of Anti-A₁ reagent and 1 volume of red cell suspension were added. B labeled test tube was placed: 1 volume of Anti-H reagent and 1 volume of red cell suspension were added. The contents in each tube was mixed thoroughly and centrifuged for 20 seconds at 1000 relative centrifugal force (rcf). The red cell button was re-suspended gently and read macroscopically for agglutination [14]. Positive: Agglutination of red cells constitutes a positive test result and indicates the presence of A₁ antigen in tube A and the presence of H antigen in tube B. Negative: No agglutination of red cell constitutes a negative result and indicates the absence of A₁ antigen in tube A and the absence of H antigen in tube B respectively.

2.10 Statistical Analysis

The data obtained was entered into Microsoft Office Excel 2007 and analysed using Statistical

Package for Social Sciences (SPSS) version 20. Chi-square and Analysis of Variance (ANOVA) were used to make comparison between variables. The results were expressed as percentage and frequency and presented in tabular form and p-Value of ≤ 0.05 was considered statistically significant.

3. RESULTS

A total of one hundred and seventy-six blood samples were collected from blood donors attending Specialist Hospital Sokoto with mean age 30.44 ± 8.210 years.

Table 1 shows the socio-demographic characteristics of the Study Subjects. Majority were within the age range of 21-30 years 89(50.6%) and > 50 years 3(1.7%) of age were the minority. The distribution of study subject based on ethnicity reveals that Hausa were the predominant with 116 (65.9%), then Fulani with 41 (23.3), Yoruba and others were the least with 13 (7.4%) and 6 (3.4) respectively. The others are Igbo, Zabarmawa, Dakarawa and iber. The distribution of study subject based on gender shows that Male donors were predominant with 171 (97.2%) while females were few with only 5 (2.8%). The distribution of study subject based on occupation shows that students were the majority with 84 (47.7%), 63 (35.7%) were civil servant, 22 (12.5%) were business and 7 (4.0) for others.

Table 2 reveals the Prevalence of ABO and Rh D among study subjects. Blood group O+ was the most frequent group with 93 (52.8%), 39 (22.2%) were blood group B+, 37(21.0%) were blood group A+, 5 (2.8%) were blood group AB+, 2 (1.1%) were blood group O-. No data were obtained for A-, B- and AB- blood groups.

Table 3 shows the Distribution of ABO blood group based on ethnic groups among study subjects. Majority of the subjects with A, B, AB and O blood groups were Hausas of 29 (78.4), 20 (51.3), 3 (60.0) and 63 (66.3) respectively, Fulani were the second and Yoruba were the third and others were the least. The p-value obtained shows there is statistically significance difference between ethnic groups (Hausa, Fulani and Yoruba) and A blood group ($p < 0.05$). While B, AB and O blood groups are statistically insignificant ($p > 0.05$).

Table 4 shows the Distribution of ABO group system based on Gender among study subjects.

There is no statistically significant difference between ABO blood groups and gender ($p > 0.05$). Table 5 shows the distribution of ABO, Rh D and subgroups of A (A_1 and A_2) and AB (A_1B and A_2B) among blood donors. Blood group O+ was the most frequent group with 93 (52.8%), 39 (22.2%) were blood group B+, 32(18.2%) were blood group A_1+ , 5 (2.8%) were blood group A_2+ , 5 (2.8%) were blood group A_1B+ , 2 (1.1%) were blood group O-.

4. DISCUSSION

The ABO blood group system is the most clinically significant blood group system because of the regular occurrence of antibodies of the blood group system and ability of antibodies of the systems to cause haemolytic transfusion reaction and haemolytic disease of fetus and new born (HDFN). The prevalence of ABO blood group varies from race to race [6].

Table 1. Socio-demographic characteristics of the study subjects

Demographic characteristics	N (%)
Age group (years)	
21-30	89 (50.6)
31-40	67 (38.1)
41-50	17 (9.7)
>51	3 (1.7)
Age (Mean± SD)	30.44±8.210
Gender	
Male	171 (97.2)
Female	5 (2.8)
Ethnicity	
Hausa	116 (65.9)
Fulani	41 (23.3)
Yoruba	13 (7.4)
Others	6 (3.4)
Occupation	
Student	84 (47.7)
Civil Servant	63 (35.7)
Business	22 (12.5)
Others	7 (4.0)

Key: N = Number of Subjects, % = Percentage, SD = Standard deviation

Table 2. Distribution of ABO, Rh D among study subjects

Blood group	Frequency	Percentage (%)
A+	37	21.0
B+	39	22.2
AB+	5	2.8
O+	93	52.8
O-	2	1.1
Total	176	(100)

Key: += RhD positive, - = Rh D negative

Table 3. Distribution of ABO blood group based on ethnic groups

Ethnicity	A	B	AB	O
	N (%)	N (%)	N (%)	N (%)
Hausa	29 (78.4)	20 (51.3)	3 (60.0)	63 (66.3)
Fulani	6 (16.2)	12 (30.8)	1 (20.0)	23 (24.2)
Yoruba	2 (5.4)	4(10.3)	0 (0.0%)	7 (7.4)
Others	0 (0.0)	3 (7.7)	1 (20.0)	2 (2.1)
p-Value	0.01	0.09	0.306	0.577

Key: N= number of subjects, % = percentage within the ABO blood group

Table 4. Distribution of ABO blood group based on gender

Gender	A N (%)	B N (%)	AB N (%)	O N (%)
Male	37 (100.0)	38 (97.4)	4 (80.0)	92 (96.8)
Female	0 (0.0)	1 (2.6)	1 (20.0)	3 (3.2)
p-value	0.333	0.324	0.62	0.448

Key: N= Number of study subjects, %= percentage within ABO blood group

Table 5. Distribution of ABO, Rh D and subgroups of A (A₁ and A₂) and AB (A₁B and A₂B) among blood donors

Blood group	Frequency	Percentage (%)
A ₁ +	31	17.6
A ₂ +	6	3.4
A ₁ B+	5	2.8
O+	93	52.8
O-	2	1.1
Total	176	(100)

Key: += Rh D positive, - = Rh D negative

In this study, it was observed that 54.0% of subjects were blood group O, 22.2% were blood group B, 21.0% were group A and 2.8% were group AB. The study also reveals blood group A was more common than blood group B among Hausa speaking language. The finding is in line with previous reports by Abubakar et al. [11] and Erhabor et al. [15] who reported a significantly higher number of blood group O individuals and predominance of allele B over allele A among the participants in their study. The findings are however at variance with some reports in other regions of Nigeria; Erhabor et al. [16], Jeremiah [17], Worledge et al. [18], Falusi et al. [19] and Omotade et al. [20] which investigated the prevalence of ABO and Rhesus D blood groups and obtained a prevalence pattern (O > A > B > AB) among student of African descent in Port Harcourt, among students in the Niger Delta, among the Yoruba and Hausa ethnic groups, in five zone of Nigeria and in Ibadan respectively. The high prevalence of O blood group observed in this study among the blood donors attending Specialist hospital Sokoto in North Western Nigeria seems an advantage particularly in terms of optimizing the use of scarce blood resource by potentially utilizing the blood group O stock against ABO blood group barriers for patients of other blood groups (A, B and AB) particularly in emergency situations. Blood group O individuals lack ABO blood group antigens on their red cell and thus are termed universal donors. Such blood can be transfused to patients of blood groups A, B and AB. However, there is a caveat

to this rule and some level of caution need to be exercised. This is particularly true because the plasma of some blood group O blood individuals has been shown to contain high titer of potent A and B immune haemolytic antibodies (haemolysins) that can potentially cause the haemolysis of red cells containing antigen A and B. Evidence –based best practice in the developed world advocate for the routine testing of all blood group O donor blood for the presence of these α and β haemolysin and that those containing high titer haemolysin should be reserved specifically for group O patients only. Those samples which are negative for high titer haemolysin could be given to groups A, B, and AB individuals in emergency situations, when ABO group specific units are not immediately available.

The distribution of ABO blood groups based on tribe shows there was statistically significant difference between “A” blood group and ethnic groups. Out of 176 donors enrolled, 115 (65.3%) were Hausa, 42 (23.8%) were Fulani, 13 (7.4%) were Yoruba and 6 (3.4%) others. This could be due to the fact that the area where the research was conducted is Hausa and Fulani dominated community. The study reveals the majority of blood donors were male. Among 176 subjects, 163 (92.6%) were male and 13(7.4%) were female donors. This study is in line with Abubakar et al. [11], who reported a significantly high number of male donors (98.8%) compared to female donors (1.18%) in their cohort study conducted in Usmanu Danfodiyo University Teaching Hospital Sokoto. This male gender participation in blood donation than females could be based on the belief that men have more blood volume and are healthier than women because women shed blood monthly, due to pregnancy and also breastfeeding and female’s belief that they don’t have enough blood for them to donate to the persons in need.

Out of the 176 study subjects, 37 (21.0%) were blood group A and 5 (2.8%) were AB blood group. Of 37 (21.0%) blood group A, 31 (83.8%)

were A1 antigen 6 (16.2%) were A2 antigen. Of the 5 "AB" blood group, 5 were all A1B. A2B was not found and this may be due to limited number of "AB" blood donors tested in the study. The occurrence of A1 and A2 obtained from this study is at variance with Sujata et al. [5], who conducted a research of which 20,864 blood donors were analysed of 5466 (26.20%) of A group, 5406 (98.90%) belonged to A1 subgroup and only 60 (1.10%) belong to A2 subgroup. Of 1708 donors with blood group AB, 1532 (89.70%) belong to A1B subgroup and 176 (10.30%) belong to A2B.

The Rhesus blood group system is the second most clinically significant blood group system after the ABO blood group system. Rh incompatible transfusions are also potentially detrimental to health. In this study, it was observed that the prevalence of Rh D positive and negative among blood donors were 98.9% and 1.1% respectively. The finding of this study is consistent with the study conducted by Erhabor et al. [15] in Zamfara State in North Western Nigeria, who studied 500 subjects, 494 (98.8%) were Rh D positive while 6(1.2%) were Rh D negative. This study is also in line with the study conducted by Abubakar et al. [11] where a total of 8,975 blood donors were studied and the Rh D distribution were 8,657 (96.46%) and 318 (3.54%) for Rh D positive and Rh D negative respectively. The study is also in line with Erhabor and colleagues [16], who conducted a research in Niger Delta of Nigeria, were 93% of their subjects were Rh D positive while the remaining 7% of the study population were negative. The percentage of Rh D negative observed in this study (1.1%) is significantly lower than the prevalence rate of >14% Rh D negative phenotype observed in study conducted by Cerny et al. [21] among Caucasians. This could be due differences in individual genetic makeup and ethnicity.

5. CONCLUSION AND RECOMMENDATION

In conclusion, the distribution of ABO obtained among study subjects shows a pattern of O>B>A>AB with the exception of Hausa ethnic group who had higher prevalence of blood group A than blood group B. This study shows that majority of the subjects had higher A₁ antigen than A₂ antigen. The study also reveals that most of the blood donors were male. This study has shown that there was statistically significant difference between blood group A and ethnic groups. There was no statistically significant

difference between ABO blood group and gender. ABO subgroups (A₁, A₂) should be included in donor screening criteria to avoid any possible transfusion reaction in Nigeria.

CONSENT

Subjects recruited into the study were requested to give a written informed consent prior to the sample collection by filling a standard informed consent form.

ETHICAL APPROVAL

The ethical approval for the study was obtained from the Ethics and Research Committee of Specialist Hospital, Sokoto.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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