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RESEARCH ARTICLE

THE PREVALENCE AND DISTRIBUTION OFABO AND RH-D BLOOD GROUPS AMONG DIFFERENT COMMUNITIES OF UNITY STATE, SOUTH SUDAN

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ABSTRACT

Background: Understanding the distribution of ABO and Rh-D blood groups in a local population is crucial for planning and managing blood bank inventories and ensuring safe blood transfusion practices. Despite such importance, there is no data in South Sudan on the distribution of the ABO and Rh-D blood groups in the population across the country. ***Methods:*** Using a conventional forward blood typing approach, we examined the prevalence of ABO and Rh-D blood groups and their distribution among different community members from seven ethnic counties of Unity State living in a Protection of Civilian (PoC) site in Juba, South Sudan. ***Results:*** The results revealed that, out of the 339 participants, blood group O was the most prevalent (57.5%), followed by blood groups A (21.8%), B (15.9%), and AB (4.7%), across all the study areas. Rh-D-positive participants comprised 89.1% of the study population, while Rh-D-negative participants made up 10.9%. When the ABO and Rh-D blood group distributions were analyzed with respect to the participants' areas of origin and gender, the study revealed no significant association between blood group distribution and area of origin. However, there was a significant difference in the distribution of the ABO and Rh-D blood groups between male and female participants. ***Conclusion:*** This study's findings will guide future national health policy planning of national and regional blood banks in South Sudan. We recommend that a large regional or nationwide study involving participants from different regions and ethnic backgrounds be conducted in the country to confirm our findings.

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INTRODUCTION

Human blood contains a remarkable variety of essential molecules, particularly on the surface of red blood cells (RBCs), which play crucial roles in transfusion safety, clinical practice, and susceptibility to diseases (1). The surface of red blood cells contains several carbohydrate, glycoprotein, and glycolipid molecules, referred to as antigens, whose presence or absence forms the basis for determining blood group systems (2-4). To date, the International Society of Blood Transfusion (ISBT) has recognized over 30 different blood group systems, with the ABO and Rhesus (Rh) being the most researched blood groups in human populations (5). The understanding of these blood-grouping systems has significantly revolutionized modern-day transfusion and blood-grouping medicine since they were first described by Karl Landsteiner in 1901 and later by Landsteiner and Weiner in 1941. At the molecular level, the ABO blood group system is controlled by a single gene consisting of three alleles (I^A, I^B, and I^O) at the ABO locus located on the long arm of chromosome 9q (3, 6, 7). Each of these alleles encodes a different form of glycosyltransferase enzyme that adds specific sugar molecules to a carbohydrate structure known as the H antigen on the membrane of red blood cells and most epithelial and endothelial cells (2, 7), resulting in the expression of different blood group antigens (A, B, AB, or none). During the expression of the ABO gene, the A allele codes for an enzyme that adds N-acetyl galactosamine to the H antigen, the B allele codes for an enzyme that adds D-galactose, and the O allele, which occurs so frequently in the human population, carries a human-specific inactivating mutation that produces a nonfunctional enzyme, leaving the H antigen on the RBC surface unmodified (6, 8). The combination of the three ABO alleles and their corresponding antigens results in four major blood groups (phenotypes), known as A, B, AB, and O, which are frequently tested for blood transfusion, genetic studies, organ transplant, forensic pathology, anthropology, and medico-legal issues such as paternity testing. Like the ABO blood group system, the Rh system is the most polymorphic and multiantigenic blood group system and consists of more than 50 antigens within three pairs of closely linked allelic genes located on chromosome 1 (3, 8, 9).

Among all the Rh antigens, the D antigen (Rh-D) is the most medically significant antigen of this blood group, and its presence or absence on RBCs determines whether the Rh-D blood group is positive or negative (10). During blood transfusion or organ transplantation, when an Rh-D-negative person is transfused with Rh-D-positive blood or an Rh-D-negative mother carries an Rh-D-positive fetus, the immune system recognizes the antigen as a foreign agent and produces antibodies, resulting in potentially fatal haemolytic complications and diseases in the fetus/newborn (7, 9). Hence, knowledge of ABO and Rh blood group distributions in a population is critical for reducing maternal, neonatal, and other transfusion-related mortality rates through effective management of blood bank inventories. This information can also help healthcare providers anticipate, plan, and manage potential complications resulting from mismatched transfusions of blood and blood products (11).

The ABO and Rh blood group systems are the same in all human populations, but they differ in frequency and distribution due to racial, ethnic, and socioeconomic factors (3, 4, 7). Several studies have investigated the prevalence of ABO and Rh blood groups across different populations, including in Ghana, Uganda, Ethiopia, Tanzania, Saudi Arabia, Libya, and Yemen. The results showed similar distribution patterns in all countries, with the O blood group being the most predominant, followed by the A, B, and AB blood groups being the least common. Similarly, the majority of the participants were Rh-positive in all the included studies (2-5, 12, 13). Despite the significance of the ABO and Rh blood group distributions knowledge in improving healthcare services through effective management of blood banks, there are no existing data on the distribution of these blood groups in South Sudan, where blood shortages are endemic in all hospitals. South Sudan's 2016-2026 national health policy includes the development of national blood transfusion services and the establishment of regional blood banks to improve blood collection and the management of safe blood transfusion services in the country (14). Our study examined the distribution of ABO and Rh-D blood groups among members of seven ethnic Counties of Unity State in South Sudan who are living in the Protection of Civilian (PoC) site in Juba. We hypothesized that the findings of our study will influence future national health policy planning and guide the establishment and management of national and regional blood bank inventories in South Sudan.

METHODS AND MATERIALS

Study area and population: This study was conducted in Juba, the capital of South Sudan, targeting members of seven ethnic counties of Unity State living in the Protection of Civilian (PoC) site. The Juba PoC site is located outside of Juba and was originally established by the United Nations Mission in South Sudan to shelter conflict-affected populations, mainly from Unity State, seeking sanctuary in 2013. This study site was chosen because it was a single location with different communities from Unity State, and it was necessary to provide a representative insight into the distribution of the ABO and Rh-D blood groups. Additionally, conducting the study at this site was critical for participants to know their blood groups, which is required when obtaining national identity documents in South Sudan. Participants were recruited on voluntary basis following a clear explanation of the study objectives and their health significance to each member of the community. Data regarding age, sex, and place of origin were recorded on the study data collection forms.

Determination of Blood Groups: The ABO and Rh-D phenotyping was performed using conventional forward blood typing procedures based on an open slide agglutination test method as previously described by Bhasin and Chahal (15). Blood samples from each participant were aseptically collected by scrubbing the finger with a piece of cotton saturated with 70% alcohol and then piercing it with a sterile disposable safety lancet. One drop of the oozing whole blood was placed in triplicate on a clean glass microscope slide, and then a commercially available standard monoclonal IgM antisera (anti-A, anti-B, and anti-D) reagent was added in equal amounts to each triplicate sample per slide and mixed well using an applicator stick according to the manufacturer's (Meril Diagnostic Pvt Ltd, India) instructions.

After 2 minutes of incubation at room temperature, the slides were examined for agglutination reactions to each of the reagents by visual observation using naked eye. The final ABO and Rh blood groups for each sample were determined based on the presence or absence of agglutination. To maintain the accuracy of the results, we used blood group analysis images adopted from (16) as a guide to ensure that the conclusions were consistent. The ABO and Rh-D blood group data were then recorded appropriately on a standard form designed for the study.

Data entry and analysis: The ABO and Rh-D blood group data were collated and entered into Microsoft Excel 2023 for analysis. A descriptive statistical analysis was performed to determine the frequency distribution, in number and percentage, of ABO and Rh blood groups among blood donors. A chi-square test was conducted to test for differences in ABO and Rh blood group distributions by sex and place of origin of the participants using the statistical formula below, and a p-value less than 0.05 was considered to indicate statistical significance at the 95% confidence level.

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Where,

χ^2 = chi squared

O_i = observed value

E_i = expected value

Ethics Approval: Ethical approval for this study was granted by South Sudan National Ministry of Health Research Ethics Review Board (MOH-RERB) with reference number MOH-RERB 28/2023. Additional approval was granted by the School of Applied and Industrial Sciences, University of Juba Research Topics Review Committee (RTRC). Permission to conduct the study in the target area was also granted by the Camp Management Committee of Juba 3 Civilian Protection site for the study to be conducted. Additionally, a written consent to participate was also obtained from all study participants including from guardians of minor participants. Each participant was then informed about his/her blood group type after analysis of each blood sample. The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Sociodemographic characteristics of the study participants: In our study, a total of 339 volunteers from seven different ethnic counties were included to determine the distribution and frequencies of ABO and Rh-D blood groups. Of the total study participants, the majority were male 66.4% (225) and 33.6% (114) were female, and their ages ranged from 10 to 50 years. The most common age group among the participants was 10-20 years 65.2% (221), followed by 21-30 years 26.5% (90), and the least common age group was 41 years and above (2.4%, 8) (Table 1). All the adult participants consented voluntarily to participate in the study, while those regarded as minors had their consent granted through parents/guardians who showed interest in the inclusion of their children in the study to determine their blood groups.

ABO and Rh-D blood group distributions and their correlation with sex and place of origin: The analytical results for the distribution of ABO and Rh-D blood groups showed that blood group O was the most prevalent blood group (57.5%, 195), followed by blood group A (21.8%, 74), blood group B (15.9%, 54), and blood group AB (4.7%, 16). Moreover, Rh-D-positive participants were more prevalent (89.1%, 302) than Rh-D-negative participants (10.9%, 37) (Table 2).

A detailed investigation of the distribution frequencies that combined the ABO and Rh-D blood groups revealed that O-positive blood group participants were the most dominant (51.6%), followed by A-positive (19.5%), B-positive (14.2%), O-negative (5.9%), AB-positive (3.8%), A-negative (2.4%), B-negative (1.8%), and the AB-negative (0.9%) participants (Table 3). Among the seven ethnic counties, blood group O was the most prevalent, the majority of whom were O-positive. With the exception of Mayom county, which has the highest prevalence of the AB blood group, there was an even distribution of most of the blood groups in the rest of the counties, including Guit and Rubkona (A-positive, B-positive, and AB-negative) and Koch and Mayom, Leer and Mayiandit (B-positive). There was no significant difference in the distribution of ABO and Rh-D blood groups in regard to the area of origin of the study participants (χ^2 = 55.71, df= 42, *P-value* =0.08). In both sexes, blood group O was the most prevalent with 141(63%) and 54(47%) of the males and females respectively. The least prevalent blood group was group AB, which represented5.8% of the male participants and 2.6% of the female participants. The distribution of the ABO and Rh-D blood groups between males and females was statistically significant (χ^2 =15.70, df = 7, *P-value= 0.03*) (Table 3).

DISCUSSION

According to the World Health Organization and other humanitarian health organizations, South Sudan continues to experience critical blood shortages for transfusion services in all hospitals across the country. Decades of conflicts in the country have led to the destruction of both health infrastructures and the entire health system. Several regional and global studies have shown that for blood banks to effectively manage their inventories, support adequate blood supply, and provide safe blood transfusion services, knowledge of the distribution pattern of ABO and Rh-D blood groups in a particular population is essential to help healthcare practitioners predict the prevalence of ABO and Rh-D blood groups and avoid critical morbidities and mortalities resulting from blood transfusion (2, 7, 3, 9, 17, 18). To the best of our knowledge, this study is the first to be conducted in South Sudan. In this study, we report the distribution and frequencies of ABO and Rh-D blood groups among members of seven ethnic counties of Unity State, namely, Guit, Koch, Leer, Mayiandit, Mayom, Panyijar, and Rubkona. Of the 339 participants involved in the study, majority of participants were males (66.4%), while females were minority (33.6%). This finding is consistent with other previous studies in Africa, including Uganda (3), Tanzania (8, 9), Ethiopia (10, 12, 18), and Libya (5), which reported similar demographics. Although most of these studies linked low female participation to women's health-related issues such as low haemoglobin levels, menorrhagia, anaemia, postnatal blood loss, pregnancy, and breastfeeding, our study aligns with (19) and (20), who claimed that, in most countries, women are culturally believed to be physically weak, as many communities in South Sudan, including those included in this study, have strong cultural beliefs and norms. Our study also aligns well with other previous studies showing that the population younger than 25 years is the most dominant age group (9, 12, 18). The high response from young people to participate in this study suggests that the motivating factor might be a desire to know their blood groups because such information is required by the government when processing national identity documents in South Sudan.

Several studies have reported the distribution patterns of ABO blood groups within a given society, country, or region. In this study, our results were inconsistent with those of similar studies conducted in Nepal and India, which showed that blood group B was the most dominant (21-24), and in Morocco, Iran, Turkey, and Pakistan (25-28), which found blood group A to be the most dominant. We found that blood group O (57.5%) was the most dominant ABO blood group, followed by group A (21.8%) and B (15.9%) groups, while the AB (4.7%) group was the least common blood group (Table 2). Similar findings were previously reported in different parts of Africa (2, 9, 17, 18), in which blood group O was the most common, followed by blood group A, blood group B, and blood group AB; these findings are consistent with the regional trend of ABO blood group distribution and frequencies. When an Rh-D-negative individual receives Rh-D-positive blood and vice versa during a blood transfusion or organ transplant, a fetal mismatch can occur, which may cause a haemolytic transfusion reaction or haemolytic disease in the newborn (9). Hence, the determination of Rh-D status in a population is crucial in clinical practice for the effective management of blood transfusion and the prevention of any blood transfusion-related fatalities. Our study revealed that most of the participants were Rh-D positive (89.1%), and only 10.9% were Rh-D negative. This finding is also consistent with several other studies that reported a greater proportion of Rh-D-positive individuals than Rh-D-negative individuals (2, 3, 5, 9, 12, 13, 17, 18). The distribution and frequencies of ABO and Rh-D blood groups vary globally, even among members of the same ethnic group. When the ABO and Rh-D blood groups were considered together, this study revealed no significant difference in the distribution of ABO and Rh-D blood groups across all seven counties in Unity State of South Sudan (chi-square = 55.71, *P-value* = 0.08), as shown in Table 3. This finding is consistent with other regional studies that reported no significant association between ABO and Rh-D blood groups with geographical distribution of the study population (8, 17).

Table 1. Socio-demographic characteristics of the study participants (N=339)

Variables	Categories	Guit	Koch	Leer	Mayiandit	Mayom	Panyijar	Rubkona	Frequency (n)	Percentage (%)
Sex										
	Male	33	30	55	33	25	24	25	225	66.4
	Female	10	13	30	10	16	18	17	114	33.6
Age (years)										
	10 - 20	23	32	55	26	29	32	24	221	65.2
	21 - 30	14	10	20	12	12	10	12	90	26.5
	31 - 40	3	1	8	4	0	0	4	20	5.9
	≥41	3	0	2	1	0	0	2	8	2.4

Table 2. ABO and Rh (D) Blood Group Distribution among the study participants

ABO and Rh-D Blood Group	Guit	Koch	Leer	Mayiandit	Mayom	Panyijar	Rubkona		
								Cumulative (n)	Percentage (%)
A	8	7	22	8	10	11	8	74	21.8
B	6	10	8	7	8	10	5	54	15.9
AB	2	0	2	2	7	0	3	16	4.7
O	27	26	53	26	16	21	26	195	57.5
Rh (D) Positive	36	36	73	40	41	38	38	302	89.1
Rh (D) Negative	7	7	12	3	0	4	4	37	10.9

Table 3. ABO and Rh-D blood group distribution among participants and the correlation with sex and origin

Place of Origin	A+	A-	B+	B-	AB+	AB-	O+	O-
Guit	7	1	5	1	1	1	23	4
Koch	5	2	8	2	0	0	23	3
Leer	20	2	6	2	2	0	45	8
Mayiandit	8	0	6	1	1	1	25	1
Mayom	10	0	8	0	7	0	16	0
Panyijar	9	2	10	0	0	0	19	2
Rubkona	7	1	5	0	2	1	24	2
Total %	19.5	2.4	14.2	1.8	3.8	0.9	51.6	5.9
Test Statistics	Chi-square: 55.71, degrees of freedom: 42, *P-value: 0.08*							
Sex	A+	A-	B+	B-	AB+	AB-	O+	O-
Male	36	4	26	5	11	2	124	17
Female	30	4	22	1	2	1	51	3
Test Statistics	Chi-square: 15.70, degrees of freedom: 7, *P-value: 0.03*							

Moreover, most of the participants in this study were O-positive (51.6%), followed by A-positive (19.5%), B-positive (14.2%), O-negative (5.9%), AB-positive (3.8%), A-negative (2.4%), B-negative (1.8%), and AB-negative (0.9%). With exception of other ethnic counties in this study, male participants in Mayom county have the highest prevalence of the AB blood group, which may be attributed to genetic variation in the study population. Contrary to the distribution by area of origin reported earlier, the distribution of ABO and Rh-D blood groups by sex was significantly different between males and females (Chi-square = 15.70, df = 7, *P-value* = 0.03), with O-positive blood group being the most common in both sexes, followed by the A-positive, B-positive, O-negative, AB-positive, A-negative, B-negative, and AB-negative groups as the least common. According to this distribution pattern, males represented 70.9%, 54.5%, 55.2%, 85.0%, 84.6%, 50.0%, 83.3%, and 66.7% of each blood group, respectively. This difference in the distribution of ABO and Rh-D blood groups might be due to geographical and evolutionary genetic variations in the study participants. Nevertheless, our observation regarding the association of ABO and Rh-D blood groups with sex is inconsistent with the findings of (3, 9, 17, 18), who reported no association between sex and ABO blood groups, but consistent with that reported by (8).

Strengths and Limitations: Critical data on ABO and Rh blood group distributions in South Sudan are not available to support effective planning and management of blood banks. This study is the first to be conducted in the country and to provide valuable insights into the distribution of ABO and Rh blood groups in the population. Nevertheless, the study was conducted on a small proportion of the population, and the conclusions might not accurately represent ABO and Rh blood distributions in the target areas of Unity State.

CONCLUSION

Knowledge about the distribution of ABO and Rh-D blood groups is very important for healthcare planning, blood bank management, and efficient blood utilization to avoid critical blood shortages and fetal mismatch accidents. This study provides insights into the distribution of ABO and Rh-D blood groups among seven ethnic counties in Unity State of South Sudan, which identified the O-positive blood group as the most prevalent and the AB-negative blood group as the least prevalent among the native inhabitants of Guit, Koch, Leer, Mayiandit, Mayom, Panyijar, and Rubkona. The findings from our study contribute directly to South Sudan's National Health Policy (2016-2026) specific objectives 3(b) on the development of national blood transfusion services and the generation of reliable information for the management and assessment of health sector performance. We recommend a larger regional or nationwide study involving participants from different regions and ethnic backgrounds in South Sudan to confirm our findings and to assess for any regional or ethnic-specific variations in the ABO blood group and Rh-D antigens

in the general population for efficient planning of healthcare services and blood banks in the country.

Ethics approval and consent to participate: South Sudan National Ministry of Health Research Ethics Review Board (MOH-RERB) and the School of Applied and Industrial Sciences, University of Juba Research Topics Review Committee (RTRC) approved this study. Permission was also granted by the Camp Management Committee of Juba 3 Civilian Protection site for the study to be conducted. Additionally, a written consent to participate was also obtained from all study participants including from guardians of minor participants. Consent for publication This article does not contain any individual person's data in any form.

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