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Distribution of ABO, RhD Blood Group among Secondary School Students at Bishops Girls Secondary School Rushere Kiruhura Southwestern Uganda

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ABSRACT

ABO and Rhesus blood group antigens are inherited genetic markers in human blood. Haemoglobin is an efficient transporter of oxygen from the lungs to the tissues and carbon dioxide from tissues to the lungs for exhalation. The study was to determine the distribution of ABO and Rhesus blood groups among secondary school female students. The blood samples of 107 secondary school students at Bishops girls' Secondary School Rushere kiruhura Southwestern Uganda between July to August, 2023 were collected and their haemoglobin genotypes were determined using HemoTypeSC™ rapid test kit and ABO and Rhesus blood groups were determined by a standard tube method. Results. The frequencies of ABO blood groups among the study population were 20 (18.5%), 24 (22.5%), 06 (5.4%) and 57 (53.6%) for blood group A, B, AB, and O respectively. Also, the distribution of Rhesus (D) positive and Rhesus (D) negative were reported as 98 (91.6%) and 9 (8.4%) each. There was no association between ABO, Rhesus (D) and Hb genotypes observed. Data revealed that Rhesus (D) positive students were statistically higher in number compared to the Rhesus (D) negative students (p=0.000). The sequence of ABO distribution among the rural population in southwestern Uganda is; O > A > B > AB. The frequency of Rhesus (D) negative is very low among secondary school students at Bishops' Girls Secondary School Rushere Kiruhura Southwestern Uganda

Keywords: blood groups, ABO blood group, Rh group, Haemolytic Disease of the Newborn

INTRODUCTION

The ABO blood is widely credited to have been discovered by the Austrian scientist Karl Landsteiner who found three different types in 1900. He was awarded the noble prize in physiology/medicine in 1930 for his work [1-3]. ABO blood groups are based on antigen that are located on red blood cells (RBC) membrane and are coded by alleles on different loci on a chromosome [4]. Individuals are classified into four major ABO blood groups namely; A, B, AB and O depending on the antigen present on their RBC surface [5].

RhD (D or RH1), originally identified in 1939, was the first clinically important blood group to be found following the discovery of ABO 39 years earlier. A phenotypic relationship between D and an antigen on human red cells detected by antibodies made in rabbits immunized with rhesus monkey red cells, led to D being inappropriately named the Rhesus antigen. A vestige of that term remains in Rh, the name of the blood group system that contains D [6]. The Rh blood group system was discovered in 1940 by Karl Landsteiner and Weiner. It consists of D, d, C, c, E and e blood group antigens [7]. The D antigen is highly considered in blood banking and transfusion medicine, and on this antigenic basis, individuals are typed as either RhD positive or negative [8-10].

The Rhesus (Rh) antigen is found on the surface of human red blood cell (RBC) membrane and the Rh blood group system is located on the short arm of chromosome number 1 (1p34/1-1p36). It is the most polymorphic and immunogenic blood group system in humans. This system has great importance in transfusion medicine because of its role in developing HDFN, HTR, and autoimmune hemolytic anemia. Of the 61 antigens present in the Rh blood group system, five antigens (CDEce) have clinical significance [10] with the ABO system and

the Rhesus (Rh) system remaining the most clinically significant blood group antigens on the red cell membrane. If the mother is RhD-negative and the fetus RhD positive, she has a potential capacity to form antibodies if exposed to fetal antigens, a process known as RhD sensitization [11-13].

RhD variants are classified for practical purposes into 3 groups: partial D, weak D, and Del alleles. Partial D variants are characterized by mutations in the extracellular domains leading to altered epitopes, and people with these variants may develop anti-D when exposed to wild-type D antigen from transfusion or during pregnancy. Women who have partial D variants and have developed anti-D are at risk for HDFN. In contrast with partial D, weak D variants have a reduced number of qualitatively normal D antigens resulting from mutations within the intracellular or transmembrane regions of RhD. RBCs with Del alleles express low quantities of D antigen but type as D-negative on routine testing [14].

The weak D variant results from a single point mutation in the transmembranous or intracellular region of the RHD gene and is reflected by reduced quantities of the normal D antigen. The partial D variant is as a result of mutation in the extracellular regions and replacement of RhD exons by the RHCE counterparts, leading to an altered or new epitopes. Individuals with partial D contain normal number of the D antigens but with a reduced quantity of D-specific epitopes on the RhD proteins. The DEL D variant expresses very low quantities of the RhD antigen [13].

In white Europeans and Americans, the D-negative phenotype is caused by the complete deletion of the RHD gene. In Africans, the D-negative phenotype is due to the formation of RHD-CE-D hybrid gene RHD pseudogene: a premature termination codon and with the presence of an extra 37 base pair duplication. The Del phenotype, which is the result of mutations in the RHD gene exons, is found in 10% to 30% of Asians [14].

HDFN is an important cause of neonatal morbidity and death. To reduce the incidence of HDFN and mortality among fetuses and neonates, anti-D immunoglobulin has been tested in clinical trials 1960s. Anti-D immunoglobulin has been used to prevent postpartum disease in RhD-negative women, and has greatly reduced HDFN-related morbidity as well as fetal and neonatal mortality [14].

METHODOLOGY

Study design

The study employed a descriptive cross sectional study design.

Study area

The study was conducted at Bishops Girls Secondary School in Rushere, Kiruhura District.

Study population

The study population was secondary students of the above-mentioned school.

Sample size determination

Sample size was determined using Kish Leslie method of 1965.

Formula, n= $\frac{z^2pq}{d^2}$

Where

Z= 1.96

p = The prevalence of RhD variant phenotypes in Uganda, which is 0.7% according to a study done [7] in Northern Uganda

q= 1-p

d = level of significance. 0.06951

$$n = \frac{(1.96)^2 x\ 0.007(0.993)}{0.05^2}$$

n **=107**

Inclusion criteria

All students that consented were allowed to participate in the study.

Study Exclusion

Students that consented, but they had phobias of blood draw.

Data collection

In this study, data on demographic characteristics of age, sex, and ethnicity were collected via a questionnaire.

Collection of Sample

4 ml blood were collected from each participant in a disposable syringe after cleaning the puncture site with 70% alcohol pad and taking all the aseptic precautions. Blood was dispensed in an EDTA vacutainer for determination of the ABO and RhD phenotype.

Processing of Sample

In a test tube containing normal saline, EDTA blood will be added, and this mixture will then be centrifuged at 2500 rpm for five minutes. Supernatant will be discarded, and this procedure will be repeated twice or more in the same manner. After three washings of RBCs with normal saline, use button and prepare the 5% saline suspension of RBCs by adding 95 % of saline to the 5% of RBCs. This cell suspension will be used for forward Rh blood grouping.

Test Procedures

Tube Method for Rh Grouping

Tube method will be employed over Tile method because of its advantages as it allows long incubation without any drying, and the centrifugation enhances antigen antibody reaction. This method is better than Tile method for doing rapid Rh grouping. The Rh D grouping is defined by the presence of 'D' antigen on the surface of the red blood cells and by the simultaneous presence of anti D antibodies in the serum (whatsoever antigen is absent from the cells). The erythrocyte antigens were known by ANTI-D, by forward typing. Human red blood cells possessing 'D' antigen agglutinated in the presence of antibody directed towards the corresponding antigen. Sample collected in EDTA vial was proceed immediately, and if testing was delayed, blood samples were stored at 2-8^{o}C in refrigerator and examined before 48 hours of collection. Non-haemolysed samples should be used for testing. Anti D antisera were stored in 2-8^{o}C kept outside till it attains room temperature before use. All aseptic precautions were taken like cleanliness of test tubes etc. Other material required were test tubes (10×75 mm), Pasteur pipettes, normal saline, beakers, centrifuge, timer, and mixing sticks.

Techniques: **Cell grouping or forward grouping**

Test tubes were taken; and labeled with participants' number and one drop of corresponding Anti D was added to each tube. One drop of 5% red cell suspension of washed RBCs (to be tested) was added to each tube. Antibodies and cell suspension were mixed properly, incubated for 10 minutes at room temperature and then centrifuged at 1000 rpm for one minute. Tubes were gently shaken to dislodge the pellet. If the red cells separated in one or more clumps, the reaction was positive. If shaking gave a homogenous suspension again the reaction was interpreted as negative. All doubtful tests were checked by microscopy for agglutination.

Statistical analysis

Data collected from the laboratory test was recorded in -a data record sheet that were organized into column of serial number of ABO and RhD status. Data was presented in tables as frequencies of the different ABO and RhD blood group status.

Ethical considerations

The research protocol was reviewed and approved by Dean of the School of Allied Health Sciences of Kampala International University-Western Uganda. Permission to recruit students was obtained from the principal education officer of the district and Head teacher.

RESULTS

The distribution of ABO blood group among 107 samples collected and analysed of study population are: Blood group O were 57(53.6%), Blood group B were 24 (22.5%), Blood group A were 20 (18.5%) and Blood group AB were 12(5.4%). The P =0.00 shows that there are statistically significant differences between the distribution of A, B and O blood group among the study population. This is shown in table 2 below:

Table 1: Distribution of blood group among secondary school students at Bishops girls secondary school rushere kiruhura southwestern Uganda

Blood group	Number	Percentage (%)
A	20	18.5
B	24	22.5
AB	06	5.4
O	57	53.6
Total	107	100.0

KEY: A, B, AB and O are blood groups

The distribution of Rhesus (D) blood groups among 107 samples collected and analyzed of study population are: 98 ((91.4%) were Rhesus positive and 9 (8.6%) were Rhesus Negative. The data from

Rhesus grouping shows that the number of Rhesus (D) positive pregnant women is significantly higher compared to the number of the Rhesus (D) negative women (p=0.000) as shown in Table 2 below:

Table 2: Distribution of Rhesus (D) Blood Groups among secondary school students at Bishops girls' Secondary School Rushere Kiruhura Southwestern Uganda

Rhesus status	Number	Percentage (%)
Positive	98	91.4
Negative	09	8.6
Total	107	100.0

Out of 107 samples analyzed, Blood group O had the highest Rhesus negative and blood group A had none Rhesus negative. The p value (0.143) shows that there is no statistically significant difference in relationship between ABO and Rhesus (D) blood groups among the study population. This is shown in Table 3 below:

Table 3: Relationship between ABO and Rhesus (D) Blood Groups.

Parameter	Blood Group			
	A	**B**	**AB**	**O**
Rhesus				
Positive	20 (100.0)	22 (92.0)	5 (83.3)	50(88.2)
Negative	0(0.0)	4 (8.0)	1(16.7)	7(11.8)
Total	20 (100.0)	24 (100.0)	6 (100.0)	57 (100.0)

DISCUSSION

The findings of this study show that the blood group O occurs most frequently among the students and blood group AB is the least common in secondary school students at Bishops girls' secondary school rushere kiruhura southwestern Uganda Knowledge of the distribution of ABO and Rhesus blood groups is an important element in determining the direction of recruitment of voluntary blood donors as required in each region and for effective management of blood banks inventory, be it at a facility of a small local transfusion service or regional or national transfusion services. It is therefore imperative to determine and have information on the distribution of ABO and Rhesus blood group systems of different ethnic groups in any population where blood transfusion services are being offered [12]. These findings are consistent with studies done elsewhere in Africa [15]. A similar distribution of ABO blood groups has been seen in studies conducted among the Bangladesh population, Western Europeans, the in Australia by Red Cross society. However, in contrast to our study, studies conducted in India [16] and Pakistan [17]showed blood group B was the most predominant, followed by blood group O, A and AB. While another study done in Nepal by Pramanik et al. found the commonest blood group as group A, followed by blood group O, B and AB. In all the studies cited and including our study, blood group AB is the least distributed among the population of the world. This study reveals that Rhesus (D) negativity has the lowest distribution among the donors which is similar to other studies conducted on another African continent. The identification of Rhesus blood system is important to prevent erythroblastosis fetalis, which commonly arises when a Rhesus negative mother carries Rhesus positive fetus. With the low incidence of Rhesus negativity in our setting the number of cases of haemolytic disease of the newborn (HDN) are expected to be much lower.

CONCLUSION

The sequence of ABO distribution among the rural population in southwestern Uganda is; O > A > B > AB. The frequency of Rhesus (D) negative is very low among secondary school students at Bishops' Girls Secondary School Rushere Kiruhura Southwestern Uganda

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