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Distribution of Haemoglobin Variants, ABO and Rh Blood Groups in Blood Donors Attending Aminu Kano Teaching Hospital

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Abstract

Background: AfricanCommunities constitute a major part of the population prone to many haematological disorders, such as haemoglobinopathies. The frequencies of abnormal haemoglobins (Hb), ABO and Rhesus (Rh) blood groups differ from one population to another. The aim of this study was to find the prevalence of hemoglobin variants, ABO and Rh blood group distribution among blood donors in Aminu Kano Teaching Hospital. Methods: Standard cellulose acetate electrophoresis was used for the determination of Hb variants at pH 8.6, while haemagglutination techniques were used for the determination of ABO and Rh blood groups. Four hundred and twenty (420) apparently healthy blood donors comprising 418 (99.5%) males and 2 (0.5%) females with mean age of 30.29 years participated in the study. Subjects were tested for abnormal haemoglobin variants, ABO and Rhesus groups. Results: Normal haemoglobin Hb AA accounted for 304 (72.4%), followed by two other haemoglobin variants, Hb AS 114 (27.1%) and Hb AC 2 (0.5%) of the study population. The distribution of the various blood groups indicates that 42.9% (180) were blood group O, 114 (27.2%) were group A, 101 (24%) were group B while 25 (5.9%) were group AB. Rh D positivity pattern was 402 (95.7%) while Rh D negativity accounts for 18 (4.3%).Conclusion: This study showed a relatively high prevalence of hemoglobin variants in the study population. Thus, the findings can serve as part of counseling strategies which can assist intending couple in making informed decision, ultimately curb the proliferation of sickle cell trait and sickle cell disease in Nigeria.

Keywords

Blood Groups, Rhesus, Haemoglobin Variants, Sickle Cell Disease

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1. Introduction

Haemoglobins are oxygen carrying macromolecules in red blood cells. They are produced in developing erythroblasts [1]. Normal adult haemoglobin consists of four haem groups and four polypeptide chains with a total of 574 amino acids. The polypeptide chains are organized into 2α-chains and 2β-chains. Each of these chains has an attached haem group (haem moiety). The specific sequences of their amino acids are known and are important in the identification of abnormal haemoglobins involving substitution of specific amino acids [2].

Haemoglobinopathies are a diverse group of inherited disorders of hemoglobin production and function. They represent the most common single-gene disorders that are found in humans and are distributed in various frequencies

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throughout the world [1]. In general, haemoglobinopathies can be classified broadly as disorders that result from structurally altered haemoglobin molecules (e.g. sickle cell anemia) or disorders that arise from numerical imbalance of otherwise normal globin chain synthesis (e.g. β-thalassemia).

To date, over 600 known haemoglobin variants have been reported. These abnormal haemoglobins are caused by structural changes in the chains, particularly the β globin chain. Clinically significant abnormal haemoglobins that can be found in tropical countries includes: Hb S which has a wide distribution in tropical Africa, parts of India, the Caribbean, Mediterranean region, Arabian Peninsula, and elsewhere in people of African descent; Hb C is found mainly in West Africa and elsewhere in people of African descent; Hb D, particularly Hb D Punjab which is found in North West India; Hb E is found in Southeast Asia and the Indian subcontinent. Sickle β-globin chain results from a point mutation that changes the amino acid at position 6 on β-globin chain from glutamic acid to valine. The term sickle cell disease is used to describe sickle cell anaemia which is homozygous Hb SS and other double heterozygous states such as Hb SD, Hb SE, HbSOArab, Hb Sβ-thal [3]. Nigeria is the most populous Black nation that carries a heavy disease burden due to sickle cell anaemia, which affects about 2% of the population [4]. About 25% of adults in Nigeria have sickle cell trait (SCT - AS) while Hb C trait (AC) which is largely confined to Yoruba people of southwestern Nigeria occurs in about 6% of individuals. Haemoglobin S constitutes 20%-40% of the total haemoglobin in the red blood cells of sickle cell carriers (Hb AS) [4].

The membrane of the human red blood cell (RBC) is complex and contains a variety of blood group antigens, the most clinically significant being the A and B antigens. These antigens are complex oligosaccharides that differ in their terminal sugar. The antibodies against red cell antigens are called agglutinins and individuals are categorized into one of four major ABO blood groups (A, B, AB, or O) according to the presence or absence of A, B, or both antigens and agglutinins [5]. The ABO blood group system is clinically the most important in transfusion Medicine as almost all normal, healthy people older than three months of age have naturally occurring antibodies to the ABO antigens they lack [6]. ABO blood group system was first discovered by Karl Landsteiner in 1900 and was reported in 1901. Landsteiner was able to identify 3 different patterns of reactivity which he termed A, B and C. These were later reclassified as group A, B and O. Von DesCasterllo andSturli discovered group AB and reported this blood type in 1902. A, B, AB, and O represents the four major groups in the ABO system [6]. ABO blood groups are determined by the presence or absence of A or B antigens on the surface of red cells. Individuals that express the A antigens on their red cells belong to group A, those with B antigens on their red cells belong to group B, those with both A & B antigens belong to group AB, while individuals that lack the two antigens belong to group O [7].

In addition, the structure and biochemical characteristics of the ABO antigens were elucidated by many investigators [8]. The genes of ABO blood group has been determined at chromosome locus 9, and Yamamoto *et al* [9]; cloned and determined the structures of these genes. Recently, it has been made possible to analyze genetically ABO blood group antigens using molecular biology techniques [10].

The Rhesus (Rh) blood group system, on the other hand, is one of the most polymorphic and antigenic blood group systems consisting at least 45 independent antigens, the most notable are D, E, e, C, and c of which D is the most antigenic. The Rh blood group system was initially discovered over 70 years ago. This was as a result of an incompatible blood transfusion of a woman with the blood from her husband following delivery of a stillborn child with erythroblastosis fetalis. Her serum agglutinated red blood cells (RBCs) from her husband and from 80% of Caucasian ABO compatible donors [11]. This was further, described by Landsteiner and Wiener that sera from rabbits immunized with RBCs from Macaca mulatta aggulinated 85% of human RBC samples [12]. The heteroantibody was renamed anti-LW (after Landsteiner and Wiener), and the human alloantibody was renamed anti- D [13].

Unlike the ABO system, naturally occurring antibodies are not usually found in individuals who lack the various Rh antigens. Antibodies of the Rh system are alloimmune in nature with the exception of some naturally occurring anti-E antibodies [4]. The Rh D blood group is nextto ABO for its clinical significance in transfusion Medicine, is well known as primary cause of haemolytic disease of fetus or newborn (HDFN) [6]. The ability to clone complementary DNA (cDNA) and sequence genes encoding the Rh proteins have led to an understanding of the molecular bases associated with some of the Rh antigens. The order of the Rh genes on chromosome 1 is probably RHCE-RHD [14].

The incidence of haemolytic disease of the newborn (HDN) is significant in White populations because of the relatively high frequency of Rh D negative individuals that constitute approximately 15% of the population. In contrast to White people, the frequency of Rh D negativity in the Nigerian population ranges from less than 1% to about 6% in different ethnic groups across the country [4]. The highest frequency of Rh D negativity was found in the southern part of the country where frequencies of 6.01% and 5.46% have been reported in the south central and south western regions respectively. More so, the frequency of Rh D negativity is

even lower in the northern regions where frequencies of 1.44% and 0.65% have been reported in the northeastern and northwestern regions respectively [4]. On the one hand, the low frequency of Rh D negativity has resulted in the scarcity of Rh D negative RBCs in Nigerian blood banks, a situation that has made any request for Rh D negative blood a nightmare for both clinicians and blood bank staff [4].

2. Materials and Methods

2.1. Study Population

A total of 420 apparently healthy blood donors attending donors' clinic of Aminu Kano Teaching Hospital were enrolled into a study for a period of seven months; January, 2014 through July, 2014. Those blood donors were screened for ABO, Rhesus blood groups and haemoglobin electrophoresis. Of the 420 subjects, 418 were males (99.5%) and 2 (0.5%) were females with mean age of 30.29 years.

2.2. Sample Collection

Five millitre (5ml) of blood was collected aseptically by venepuncture. Two millitre (2ml) of blood was put into ethylene diamine tetra acetic acid (EDTA) anticoagulant tubes and was used for the determination of haemoglobin variants.The remaining 3ml was allowed to clot in plain tubes and was used for blood grouping (ABO & Rh blood groups). All the 420 blood donors enrolled in this study have consented to participate in the study.

2.3. Laboratory Analysis

A small quantity of haemolysate was applied on cellulose acetate paper and mounted in the electrophoresis tank containing Tris-EDTA-borate buffer at pH 8.6; the electrophoresis tank was then connected to the power pack at 350 volts and 3 - 4 mA for 20 minutes to ensure optimal separation of each band. Haemolysates of known haemoglobins (AA, AS, AC) were included as controls in each batch of test. ABO grouping was done using standard tube technique at room temperature while Rhesus grouping was done at 37°C. Blood groups were determined based on presence or absence of agglutination.

2.4. Data Analysis

Data processing was done using the Graph pad *Instat* (2007) computer statistical software package (Version 5.00) [15]. Frequency and percentage of distribution of Haemoglobin Genotype in Blood Donors were established.

2.5. Ethical Consideration

Approval for the study was obtained from the Ethical Committee of Aminu KanoTeaching Hospital, permission from the respective Head of Departments and informed consent of the participants were also obtained.

3. Results

Out of 420 blood donors tested for Hb genotype, 304 (72.4%) had Hb AA, 114 (27.1%) had Hb AS, while only 2 (0.5%) had Hb AC. None of the participants had abnormal pattern of SS or SC.

The distribution of the various blood groups indicates that 180 (42.9%) were group O, 114 (27.2%) were group A, 101 (24.0%) were group B, while 25.0 (5.90%) were group AB (Table 2).Rhesus positivity rate was 402 (95.7%) while negativity accounted for 18 (4.3%) of the total population studied (Table 3).

Out of the 420 subjects studied 110 (26.2%) were A positive, 98 (23.3%) were B positive, 24 (5.7%) were AB positive. On the other hand, 4 (1%) were A negative, 3 (0.7%) were B negative, 1 (0.2%) was AB negative, and 10 (2.4%) were O negative.

Among the subjects studied, group O with frequency of 140 (33.3%) had Hb AA while 40 (9.52%) had Hb AS, 80 (19.05%) of group A had Hb AA while 34 (8.1%) had Hb AS. Group B subjects with frequency of 74(17.62%) had Hb AA, 25 (5.95%) had Hb AS, and 2 (0.48%) had Hb AC. In AB blood group, 11 (2.62%) Hb AA, while 14 (3.33%) had Hb AS.

Table 1. Distribution of Haemoglobin Genotype in Blood Donors.

Genotype	Frequency	Percentage (%)
AA	304	72.4
AC	2	0.50
AS	114	27.1
Total	420	100

Table 2. Distribution of ABO Blood Groups in Blood Donors.

Blood group	Frequency	Percentage (%)
A	114	27.2
B	101	24
AB	25	5.9
O	180	42.9
Total	420	100

Table 3. Relationship of ABO & Rh Blood Group.

ABO blood group	N	Rh 'D' positive	Rh 'D' negative
A	114	110 (26.2%)	4 (1.0%)
B	101	98 (23.3%)	3 (0.7%)
AB	25	24 (5.70%)	1 (0.2%)
O	180	170 (40.5%)	10 (2.4%)
Total	420	402 (95.7%)	18 (4.3%)

Table 4. Relation between Blood groups and Hb Genotype.

Blood Group	Hb Genotype	Number	Percentage (%)
O	AA	140	33.33
O	AS	40	9.52
A	AA	80	19.05
A	AS	34	8.1
B	AA	74	17.62
B	AS	25	5.95
B	AC	2	0.48
AB	AA	11	2.62
AB	AS	14	3.33
Total		420	100

4. Discussion

The Hb electrophoresis of the majority of blood donors in this report revealed 72.4% for Hb AA genotype, while Hb AS (sickle cell trait) accounted for 27.1% with Hb AC having 0.5%. This finding is consistent with previous reports in which a prevalence of 80.32% was obtained for Hb AA among students in the Niger Delta of Nigeria [16]. The observed frequency of Hb AA is also within the normal range of 55% - 75% earlier reported for Blacks [16]. Similarly,the frequency of (AS) was reported as follows: 8% - 16% for Black Americans, 8% - 10% for White Americans, 6% - 15% for Europeans (United Kingdom, Pakistanis and Blacks), 1% - 15% for Europeans (Mediterranean), 3% - 8% for Caribbean's, 7% - 8% for people from Middle East, 15% - 30.5% for Africans, and 40.5% for West Africans, and Nigerians [5].

The red cell life span is normal in persons with SCT and hence the transfusion of such red cells is usually not associated with any adverse effect in the recipient. In SCT individuals, 40% of their haemoglobin is Hb S and the abundance of Hb A (about 60%) prevents the cells to sickle under normal physiological conditions [17]. In most countries, individuals with SCT are considered eligible for blood donation owing to the fact that their red blood cells do not sickle under normal physiological condition. There is no standard practice regarding the screening of donors or the use of SCT red blood cells (RBCs) for transfusion. Each blood centre develops its own regulation. Following the implementation of universal leukoreduction in several countries, problems associated with leukocyte filtration of SCT blood have been noted. RBC components from SCT donors often occlude white blood cell (WBC) reduction filters. In all such cases of exchange transfusion, Hb AA red cells should be used because the use of SCT red cell will slow down the rate of fall of Hb S level in the patient thereby prolonging the exchange procedure and hence retarding rate of clinical improvement [17].

The frequency of ABO blood groups varies from one race to another. In this study, we observed 42.9% of the subjects were group O, 27.2% were group A, 24.0% were group B while 5.9% were group AB, that is O >A >B >AB. Among Western Europeans, the distribution of ABO blood groups indicates that 46% are group O, 42% are group A, 9% are group B and 3% are group AB, that is O >A >B >AB. However, some studies have shown Eastern Europeans have a higher proportion (up to 40%) of group B blood, while American-Indians belong exclusively to blood group O. Black Americans generally demonstrate frequencies of O, A, B, and AB blood groups of 49%, 27%, 20% and 4%, respectively. A previous study, which focused on the Yoruba and Hausa ethnic groups in Nigeria indicated that 58% were group O, 21% were group A, 17% were group B and 2% were group AB respectively [18]. Thus, previous reports are in agreement with the results obtained in this study and confirm that group O is the predominant ABO blood group [5]. It is interesting to note that in another study to determine the frequency of ABO and Rh blood group antigens among 4,656 neonates born at a private hospital in Istanbul indicated that group A blood was detected most frequently followed by group O, group B, and group AB. While another study conducted in Pakistan indicated that group B was the predominant blood group [19]. The most frequent blood group in Saudis is O-positive. Blood group A is observed at a lower frequency relative to values from Western populations, whereas a significant increase in blood group B combined with a slight increase in blood group AB.

The gene frequencies among residents of Bangal, India with respect to ABO systems show a pattern O > B > A > AB. The high prevalence of group O individuals in nature is of great advantage because of their status as 'universal donors' as this implies availability of blood in cases of emergency. However, caution should be taken in doing this as some group O blood is known to have potent immune hemolytic antibodies (hemolysins) [19]. Routine hemolysin test on every group O blood will help reduce the risk of transfusion reaction. More so, the O phenotype has been reported to show parity-specific association with protective malaria immunity in pregnancy leading to improved birth anthropometry [20].

The frequency of Rh D antigen in the present study was 95.7% positive, while negativity accounted for the remaining 4.3% of the study population. This finding aligns with the 96.7% positive rate recorded in Igbos by Ukaejiofor *et al.,* [21]. 96.77% was reported by Jeremiah [19] in Port Harcourt, 93% by Bashwari *et al.,* [22] in the Eastern region of Saudi Arabia and 92.8% in South-west of Saudi Arabia respectively. This percentage of Rh D negative observed in our study (4.3%) is much lower than the prevalence rate of ≥14% Rh D negative phenotype observed in studies among Caucasians [5]. Based on this report, it can be inferred that Rh positivity has a very

high prevalence in the population studied, while Rh negativity has a very low prevalence. This is in agreement with previous studies done in different parts of the country [4, 23].

5. Conclusion

The relationship studied between blood groups and haemoglobin genotype reveals that group O individuals have the highest prevalence of Hb AA, followed by group A, B, and AB. Likewise, the prevalence of Hb AS is highest among group O subjects, followed by group B, A, and AB. Hb AC was found only in group B individuals. Based on the results of this study, it was indicated that certain blood groups may be related with some haemoglobins like Hb AC and blood group B.

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