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ORIGINAL RESEARCH

Abnormal hemoglobin variants, ABO, and Rhesus blood group distribution among students in the Niger Delta of Nigeria

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Background: Communities in Africa constitute a major part of the population that is vulnerable to many erythrocytic hereditary and hematological disorders such as hemoglobinopathies. The frequencies of abnormal hemoglobin variants, ABO, and Rhesus blood groups vary from one population to another.

Methods: The aim of this study was to find the prevalence/spectrum of hemoglobin variants, ABO, and Rhesus blood group distribution among 204 undergraduate students of African descent in Port Harcourt in the heart of the Niger Delta geopolitical zone of Nigeria. Standard alkaline cellulose acetate electrophoretic technique using the Shandon electrophoretic tank with tris-ethylene diamine tetracetic acid (EDTA) borate buffer and hemagglutination techniques were employed for the determination of abnormal hemoglobin variants, ABO and Rhesus blood groups, respectively.

Results: Two hundred and four apparently healthy students of African descent comprising 124 males (60.8%) and 80 (39.2%) females with a mean age 24.5 ± 6.5 years took part in the study. Subjects were screened for abnormal hemoglobin variants, ABO, and Rhesus groups. Normal hemoglobin accounted for 69.1%, followed by abnormal sickle cell trait in 29.4%, and the sickle cell disease in 1.5% of the study population. The distribution of the various blood groups indicated that 46% were blood group O, 26.6% were group A, 23.6% were group B while 3.8% were group AB. Rhesus (RhD) positivity rate was 93% while RhD negativity accounted for 7%.

Conclusion: This research indicates a high prevalence of hemoglobin variants in the study population. Carrier screening and mutation identification can become the cornerstones of any prevention program for hemoglobin disorders. It can also help in the formulation of genetic counseling policies to help prospective couples make informed decisions in a bid to reduce the sickling gene pool in the Niger Delta of Nigeria.

Keywords: abnormal hemoglobin variants, ABO, Rhesus blood group, Niger Delta, Nigeria

Introduction

Abnormal hemoglobin variants are a group of autosomal recessive disorders characterized by the synthesis of a structurally abnormal globin chain. Inherited disorders of hemoglobin are the most common gene disorders with 7% of the world's population being carriers.[1] Worldwide, it is estimated that there about 240,000 healthy carriers of the sickle gene variants and 300,000 children are born with sickle cell disease (SCD) every year.[2] Sickle cell disorders are estimated to affect more than 1 in 2400 births in England.[2] The highest prevalence of the sickle cell disorders is found amongst people of African or West Indian (Caribbean) descent.[2] It may also occur in

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people from the eastern Mediterranean, Middle East, India, and Pakistan.[2] In countries located in malarious regions of the world, a few of the mutations have reached high gene frequencies because of the protection they provide against malaria.[1] Sickling disorders include the heterozygous state for hemoglobin S (HbS) or the sickle cell trait (AS), the homozygous state for HbS or sickle cell anemia (SS), and the compound heterozygous state for HbS together with other hemoglobin (C, D, E) or other structural variants. Hemoglobin S differs from hemoglobin A by the substitution of the amino acid valine for glutamic acid at position 6 in the β-chain. The prevalence of sickle cell anemia (HbSS) among the black population in the United States is reported to be 9%[3] and 30%–40% generally for Africans.[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 actually 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 and B antigens and agglutinins.[5,6] In addition, human red blood cells that contain antigen D and are known as known as Rhesus positive while those without antigen D in their RBC's are Rhesus negative. The clinical relevance of these blood group systems relates to the capacity of alloantibodies (directed against antigens not possessed by the individual) to cause destruction of transfused red cells (ABO antibodies) or to cross the placenta and give rise to hemolytic disease of a newborn (HDN).[7]

Knowledge of the distribution of the various blood groups and abnormal hemoglobin variants is vital in determining the type and stock levels to be maintained in the hospital blood bank as well as in the formulation of transfusion policies. As an example, patients with sickle cell disease are often faced with the risk of alloimmunization from allogeneic blood transfusions. Carrier screening and mutation identification, therefore, can form one of the cornerstones of any prevention program for the hemoglobin disorders. The strategy for carrier screening and mutation analysis is based on that fact that although heterozygotes are symptom free, they present specific hematologic characteristics that are useful for their identification.

The most populous country in Africa, Nigeria accounts for approximately one-sixth of Africa's people. The variety of customs, languages, and traditions among Nigeria's 389 ethnic groups gives the country a cultural

diversity. The Niger Delta of Nigeria is a densely populated oil-producing region. The Niger Delta, as now defined officially by the Nigerian Government, extends over about 70,000 km² and makes up 7.5% of Nigeria's land mass. Historically and cartographically, it consists of 9 of the 36 states in Nigeria: Bayelsa, Rivers, Delta, Abia, Akwa Ibom, Cross River, Edo, Imo, and Ondo. Some 31 million people belonging to more than 40 ethnic groups including the Efik, Ibibio Annang, Oron, Ijaw, Itsekiri, Igbo, Urhobo, and Kalabari are among some who speak approximately 250 dialects in the Niger Delta. This study aimed to determine the prevalence/spectrum of hemoglobin variants, ABO and RhD blood group distribution among 204 undergraduate students of African descent from the ethnic groups of the Niger Delta of Nigeria attending the Rivers State University of Science and Technology in Port Harcourt.

Materials and methods
Study population

Participants were selected randomly and drawn from adult students of African descent from the Niger Delta of Nigeria attending the University of Science and Technology in Port Harcourt, Nigeria. A total of two hundred four (204) participants (124 males and 80 females, aged ≥18 years, mean age 24.5 years and 95% confidence range of 18–31 years) were enrolled into this study. The study was carried out in the Laboratory Department of Omoku General Hospital. The institutional ethical committee approved the study. All the participants gave their written, informed consent and were offered pre- and post-test counseling.

Sample collection and laboratory methods

As mentioned, this study aimed to find the prevalence/spectrum of hemoglobin variants, ABO, and Rhesus blood group distribution among 204 undergraduate participants of African descent from the Niger Delta geopolitical zone of Nigeria.

Blood samples were collected by venipuncture into ethylene diamine tetracetic acid (EDTA) anticoagulated tubes and used for the determination of abnormal hemoglobin variants and red cell phenotyping. The method described by Brown[8] was used for hemoglobin electrophoresis. A small quantity of hemolysate of venous blood from each of the subjects was placed on a cellulose acetate membrane and carefully introduced into the electrophoretic tank containing tris-EDTA-borate buffer at pH 8.6. Electrophoretic separation was then allowed to operate

for 15–20 minutes at an electro motive force (emf) of 160 V. The results were read immediately. Hemolysate from blood samples of known hemoglobin (AA, AS, AC) were run as controls. Red cell phenotyping was carried out with standard tube techniques as described by Judd[9] and Brecher.[10] For ABO blood grouping, a drop each of anti-A, anti-B, and anti-AB reagents (Biotec, Ipswich, UK) was placed in clean test tubes labeled 1, 2, and 3. To each tube a drop of 5% red blood cell suspension in saline was added. The contents were gently mixed together and centrifuged for 30 seconds at 1000 g. The cell buttons were re-suspended and observed for agglutination. Agglutination of tested red cells constituted positive results. A smooth cell suspension after re-suspension followed by a microscopic confirmation constituted negative test results. For Rhesus D typing, a drop of anti-D serum (Biotec) was placed in a clean, labeled test tube and a drop of control was placed in a second tube. One drop of 5% RBC suspension in saline was then added and incubated at 37°C. At the end of the incubation period, the contents of the tube were mixed gently and centrifuged for 30 seconds at 1000 g. Agglutination was read macroscopically and microscopically in doubtful cases. All negative results were confirmed using the indirect antiglobulin test (IAT) procedure for confirmation of weak D.

Statistical analysis

Statistical analysis was analyzed using computer database software from the Statistical Package for Social Sciences (version 10; SPSS Inc., Chicago, IL) to generate frequency distribution and percentage prevalence scores of the various parameters. Descriptive analysis of the percentages of continuous variables was reported. Comparisons were assessed using mean and chi-square tests. A P-value of <0.05 was considered statistically significant in all clinical comparisons.

Results

A total of 204 participants were screened for ABO, Rhesus blood groups and abnormal hemoglobin variants. Out of the 204 apparently healthy students of African descent made up of 124 males (60.8%) and 80 (39.2%) females with a mean age 24.5 ± 6.5 years screened, 141(69.1%) subjects had (normal hemoglobin [HbAA]), 60 (29.4%) had (abnormal sickle cell trait [HbAS]), and 3 (1.5%) had (HbSS). None of the participants had abnormal pattern AC or SC. Figure 1 shows the distribution of the abnormal hemoglobin variants among the study participants. The distribution of abnormal hemoglobin variant was further compared based on gender (Table 1). Fifty eight (41.1%) males and 83 (58.8%) females had (HbAA), 23 (38.3%) males and 37 (61.7%) had (HbAS) while 1 (3%) male and 2 (6%) females had (HbSS).

The distribution of the various blood groups revealed that 66 (46%) were group O, 26 (26.6%) were group A, 22 (23.6%) were group B, while 2 (3.8%) were group AB. (Table 2). The distribution of ABO and Rhesus blood groups of the all study participants is shown in Table 2. ABO groups were further compared based on gender (Table 3). The distribution of ABO blood groups showed that 32.3% of males and 13.7% of females were group O, 12.8% and 13.8% were group A, 12.7% and 10.9% were group B while 2.9% of males 0.9% of females were group AB. Rhesus positivity rate was 190 (93%) while negativity accounted for 14 (7%) for the total population studied. The distribution of the percentage frequency of Rhesus D groups among the study participants showed that 116 (56.9%) of males
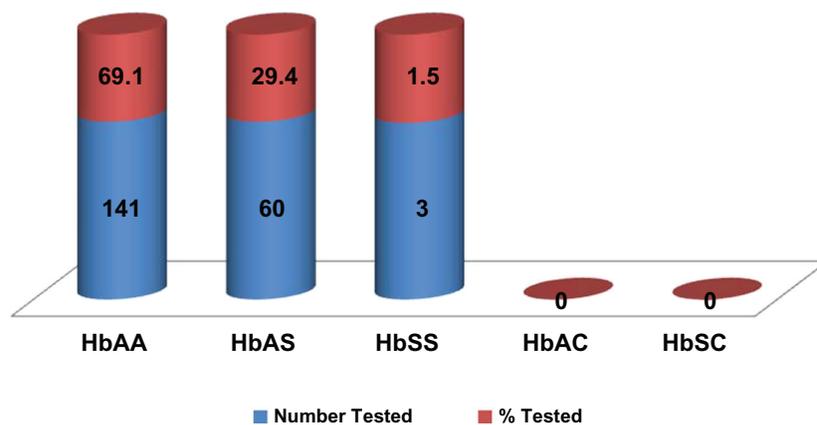

Figure 1 Distribution of abnormal hemoglobin variants among participants
Abbreviations: HbAA, normal hemoglobin; HbAC, hemoglobin C trait; HbAS, abnormal sickle cell trait; HbSC, hemoglobin SC disease; HbSS, sickle cell anemia.

Table 1 Distribution of the abnormal hemoglobin variants based on gender

Gender	HbAA (%)	HbAS (%)	HbSS (%)	HbAC (%)	HbSC (%)
Male	58 (41.1%)	23 (38.3%)	1 (3%)	nil	nil
Female	83 (58.8%)	37 (61.7%)	2 (6%)	nil	nil

Abbreviations: HbAA, normal hemoglobin; HbAC, hemoglobin C trait; HbAS, abnormal sickle cell trait; HbSC, hemoglobin SC disease; HbSS, sickle cell anemia.

Table 3 Distribution of ABO and Rhesus blood groups based on gender

Gender	Group A (%)	Group B (%)	Group AB (%)	Group O (%)	Rh-positive (%)	Rh-negative (%)
Male	26 (12.8)	26 (12.7)	6 (2.9)	66 (32.3)	116 (56.8)	8 (3.9)
Female	28 (26.6)	22 (10.9)	2 (0.9)	28 (13.7)	74 (36.2)	6 (2.9)

and 74 females (36.2%) were RhD positive. By contrast, 8 males (3.9%) and 6 (2.9%) females were RhD negative (Table 3).

Discussion

In this study on a small population from the Niger Delta of Nigeria, we observed a prevalence of 69.1%, 29.4%, and 1.5% for HbAA, HbAS, and HbSS, respectively. By comparison, the prevalence of HbSS among the black population in the United States is reported to be 9% and 30%–40% generally for Africans.[3,4] In another report the geographical distribution of SS was given as follows: 3%–9% for black Americans, 1%–8% for white Americans, 3%–7% for Europeans (United Kingdom among Pakistanis and blacks), 2%–8% for other European countries (Mediterranean), 1%–3% for Caribbeans, 1%–3% for Middle East, 1%–10% for Africans. 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 Caribbeans, 7%–8% for Middle Easterns, 15%–30.5% for Africans, and 40.5% for West Africanerns, and Nigerians.[11]

Our finding of a prevalence of 29.4% HbAS and 1.5% HbSS is consistent with previous reports among undergraduate students in Bayelsa state[12] and Rivers state,[13] both in the South-South of Nigeria where prevalence rates for HbSS of 2% and 3%, respectively, were observed. However, a study carried out in Kenya, East Africa observed a 0% prevalence of HbSS. Instead the authors observed a prevalence of 74% and 97% for HbAA in lowland and highland areas and 26% and 3% for HbAS in lowland and highland areas, respectively.[14] The zero frequencies observed in this study possibly imply that the sickling gene pool may gradually be reducing in some African populations, particularly those with an abnormal hemoglobin carrier screening and genetic counseling program for the prevention of hemoglobin disorders.

The number of people with homozygous SS in Nigeria is high. This is thought to be due to the absence of carrier testing programs or premarital counseling/testing for prospective couples prior to marriage in a bid to reduce the prevalence of hemoglobin disorders. The universal neonatal screening program is an effective way to diagnosis the presence of hemoglobinopathy. Experience in Belgium has shown universal neonatal screening to be an excellent health education tool.[15] Countries in Africa can benefit by implementing similar programs, as their development is pivotal to improving the health care of those affected by hemoglobin disorders. However, such program require major economic and organizational resources, which must be taken into account and balanced against other local health priorities.[16] There is also a need for a sickle cell disease clinical care programs which should include: infection prophylaxis with penicillin and malarial prophylaxis; family training to identify early, severe, or persistent symptoms and increase awareness of the gravity of malarial crises; the evaluation of the patient's nutritional status and fluid intake; and education about the importance of regular medical visits.

The frequency of HbAS detected in this study (29.4%) is consistent with previous studies in Nigeria and other African settings which observed a prevalence of 20%–40% in Africa, in general.[11,17,18] Hemoglobin S, in comparison with HbA, is thought to offer some protective role against Plasmodium falciparum malaria and conclusive evidence of this exists with Hemoglobin S (beta 6Glu- > Val) and HbC (HbC; beta 6Glu- > Lys), both occurring in sub-Saharan Africa. However, the mechanism(s) of the protection exerted remain(s) debatable for both hemoglobin variants HbC and HbS. Recently, an abnormal display of PfEMP1, an antigen involved in malaria pathogenesis, was reported on

Table 2 Distribution of ABO and Rhesus blood groups

ABO blood group	Number of subjects (%)	Number of subjects (%) Rh-positive	Number of subjects (%) Rh-negative
A	54 (26.5)	52 (25.5)	2 (0.98)
B	48 (23.5)	44 (21.6)	4 (1.96)
AB	8 (3.9)	6 (2.9)	2 (0.98)
O	94 (46.1)	88 (43.1)	6 (2.94)

HbAC and HbCC infected erythrocytes that showed reduced cytoadhesion and impaired rosetting *in vitro*.[16] We did not detect hemoglobin C either as homozygous HbCC, homozygous HbAC, or as doubling homozygous for two abnormal hemoglobin HbSC. However a previous study in the Niger Delta[12] reported a prevalence of a 2% and 4%, respectively, for HbAC and HbSC. Similarly, a study of 204 individuals of a black ethnic background who were migrant Africans in Spain[19] described a 3.4% prevalence rate of HbAC, while 0.5% had HbCC.

We observed a gender–associated risk for hemoglobin S trait (HbAS) and HbSS of 63.3% and 6% for females versus 41.6% and 3% for males. The reason for this female gender susceptibility for abnormal hemoglobin S is unknown.

The frequency of ABO blood groups varies from race to race. We observed that (46%) of our subjects were group O, (26.6%) were group A, (23.6%) were group B while (3.8%) were group AB (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 (O > A > B > AB). However, some Eastern Europeans have a higher proportion (up to 40%) of group B blood, while pure American Indians belong exclusively to blood group O.[18] American blacks generally demonstrate frequencies of O, A, B, and AB blood groups of 49%, 27%, 20% and 4%, respectively (O > A > B > AB).[20] A previous report which focused on the Yoruba and Hausa ethnic groups in Nigeria by Worlledge et al[21] indicated that 58% were group O, 21% were group A, 17% were group B and 2% were group AB. Previous reports are in agreement with the frequencies obtained in this study and confirm that group O is the predominant ABO blood group. However an exception to this was observed among the Gwari tribe of Abuja and the Rubuka tribe of the Plateau state of Nigeria in which group B was the predominant ABO blood group. The high frequency of group O observed in our study among the people of the Niger Delta provides an advantage in terms of availability of blood for transfusions, especially in emergencies. Blood group O individuals lack ABO blood group antigens on their red cell and thus their blood can be given to people of blood groups A, B and AB. However, some level of caution has to be exercised since the plasma of some group O blood individuals is known to contain high titer of potent A and B immune hemolytic antibodies (hemolysins). Routine hemolysin testing should be carried out on all group O blood samples to allow those containing high titer hemolysin to be reserved specifically for group O patients. Those samples which are negative for high titer hemolysin could be given to groups A, B, and AB individuals in emergency situations, where ABO group specific units are not available, to reduce the risk of transfusion reaction.

The frequency of RhD antigen in the present study was 93% while negativity accounted for the remaining 7% of the study population. This finding aligns with the 96.7% positive rate recorded by Ibos by Ukaejiofor et al[22] 96.77% documented by Jeremiah[23] in Port Harcourt, 96.6% by Pramanik et al[24] in Nepal, 94% by Mwangi[25] in Kenya, 93% by Bashwari et al[26] in the Eastern region of Saudi Arabia, and 92.8% by Sarhan et al[27] in Southwest of Saudi Arabia. This percentage of RhD negative observed in our study (7%) is much lower than the prevalence rate of ≥14% RhD negative phenotype observed in studies among Caucasians.[28,29] The obstetric implication of the low prevalence of D-negative in the Niger Delta population is that RhD alloimmunization problem may be of a much smaller magnitude than it is in most western countries. The Rhesus blood group system is the second most clinically significant red cell antigen system after the ABO blood group system. This is because a substantial proportion of the population lacks the major Rh antigen known as D. In such individuals, the likelihood of becoming sensitized to the D antigen following exposure by transfusion of RhD positive red cells or during pregnancy involving a Rhesus positive fetus is very high and the antibody D produced as a result of such immunization has serious clinical effects including hemolytic disease in the newborn and/or transfusion reactions. In this study we have observed a significantly higher prevalence of the sickling gene and a lower prevalence of RhD negative blood group compared to values reported among Caucasians.

Knowledge on the distribution of the various blood groups as well as abnormal hemoglobin variants is vital in the safe rendering of transfusion services, civic registration, and forensic medicine. As mentioned above, carrier screening and mutation identification can become the cornerstones of any prevention program for the hemoglobin disorders. It can also help in the formulation of genetic counseling policies to help prospective couples make informed decisions in an effort to reduce the sickling gene pool in the Niger Delta of Nigeria.

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Disclosures

The authors report no conflicts of interest in this work.

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