Percentage Expression of Lewis B, Rh-e, N and ABO Antigens in descents of Bonny Kingdom, River, State, Nigeria.

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ABSTRACT

Introduction: The presence of rare blood group antibodies in the blood of donors/recipients stimulate red cell alloimmunization and constitute a major cause of transfusion related reaction after a compatible ABO cross match, yet remain one of the less routinely assessed prior to blood donation and transfusion in Nigeria. This cross-sectional study was carried out among indigenes of Bonny kingdom in Rivers State Nigeria to access the percentage expression of Lewis B, Rh-e, N and ABO antigens in descent of Bonny Kingdom Rivers State Nigeria.

Materials and Methods:- One hundred and twenty (120) apparently healthy subjects consisting of sixty (60) females and sixty (60) males aged between 18-50 years all indigenes of Bonny Kingdom were recruited by structured questionnaire for the study. 4mls of venipuncture blood was aseptically collected from each participant into vacutainer bottle containing 0.5 mL of 1.2 mg/mL dipottasium ethylenediaminetetraacetic acid anticoagulant and a 5% cell suspension was prepared and used to determine the various blood groups antigens using the standard tube Agglutination technique.

Results: Results obtained shows a total of 94.1% (113) of the participants expressed Rh-e antigen, 45.8% (55) male and 48.3% (58) females. 7.5% (9) of the participants expressed the N antigen, 3.3% (4) male and 4.2% (5) females. 4.2% (5) of the study population expressed Lewis B antigen, 2.5% (3) males and 1.7% (2) female. The ABO system showed 68.5% (79) O blood group, 29.1% (35) male and 36.7% (44) female. 22(18.3%) typed for A blood group, 11.6% (14) male and 6.7% (8) female. For B blood group 14.2% (17) persons were typed, 7.5% (9) male and 6.7% (8) female while 1.6% (2) persons were typed as AB and they are all females.

Conclusion: Rh-e phenotype occurred highest among the study participants. The proportion of the population without the expression of these antigens of the blood group tested has the potential to be alloimmunized during blood transfusion and develop antibodies, some of which can be responsible for transfusion reactions and haemolytic disease of the new born. This study has provided baseline data on the distribution of some blood group antigens in

the Bonny Kingdom of Rivers State in Nigeria. Based on the finding in this study, it is recommended that Rh-e grouping be carried out on pregnant mothers, blood donors and recipients before transfusion; while Lewis B and N blood groups may be subjected to expansive population testing.

KEYWORDS: Alloimmunization, Lewis, Rh-e, N antigens, Bonny Kingdom.

Introduction

The presence of rare blood group antibodies in the blood of donors/recipients stimulate red cell alloimmunization and constitute a major cause of transfusion related reaction after a compatible ABO cross match, yet remain one of the less routinely assessed prior to blood donation and transfusion in Nigeria and particularly in Bonny Kingdom (1).

Detection of blood group antibodies in a population and making available corresponding compatible negative blood group is a sure way of preventing red cell alloimmunization and transfusion related reactions both in pregnant mothers, blood donors and recipients. The Bonny Kingdom, otherwise known as Grand Bonny is a traditional state in the town of Bonny, located in Bonny Local Government Area of Rivers State, Nigeria. In the pre-colonial period, it was an important slave trading port and later was a key location for trading palm oil products. During the 19th century the British became increasingly involved in the internal affairs of the kingdom, and in 1886 assumed control under a protectorate treaty (2).

In human plasma, Lewis antigens are found to be attached to erythrocytes, platelets and lymphocytes that are circulating by direct insertion of their lipid anchor into the plasma membrane of the above mentioned cells; while in body secretions, Lewis blood group antigens are also similarly attached to an amino acid component of the glycoprotein (3, 2). The Lewis antigens do not have their synthesis on the red blood cells, but are absorbed from plasma (3).

Lewis antibody Le^b occur as IgM in nature, it react at room temperature and can activate complement and does not cause haemolytic transfusion reaction or haemolytic disease of the newborn thus not clinically significant (3,9,2).

The Lewis agglutinogens, are biochemical structures synthesized by exocrine epithelial cells that are absorbed passively into red blood corpuscular bi-layers, and some group of Lewis agglutinogens function as counter ligands for selectins, this has been observed to be consistent with the relationship of Lewis antigens in the occurrence or development of thrombosis. Lewis-b (Le^b) is a receptor for *Helicobacter pylori* (5).

Research has shown the frequency distribution of Le^b in Whites and in Blacks as 72% and 55 % respectively (6, 7, 8). Although the Lewis antigens are weakly expressed in cord blood, their expression in human begins at 2 years old (7). An earlier study on the prevalence of Lewis A amongst indigenes of Bonny Kingdom showed that a total of 12 (10%) of the study population were positive for Lewis A blood group antigen, 3(2.5%) females and 9(7.5%) males (2).

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

antigens D, C, c, E, and e are the most important (9,10). Anti-D, anti-C, anti-E, and anti-e have all been involved in haemolytic transfusion reactions, particularly delayed reactions (11, 1, 2). The clinically important Rhesus antigens C, c, E and e are the result of RhCE protein changes at only five amino acid locations (12, 13, 14).

Report in literatures shows that the Rh antigen C, c, E, and e are not so immunogenic but are highly important in patient care upon the development of the corresponding antibody (2). Beside their importance in blood transfusion and haemolytic disease of the newborn, the physiological function played by this protein can only be speculated as been involved in the transportation of ammonium across the RBC membrane and the maintenance of the integrity of the red cell membrane (15).

The MNS nitrogen system is a human blood group system based upon two genes (glycophorin A and glycophorin B) on chromosome 4 (10). There are currently 46 antigens in the system but the five most important are called M, N, S, s, and U (16, 10). The MN blood group system is under the control of an auto social locus found on chromosome 4, with two alleles designated Lm and Ln. The blood type is due to glycoprotein present on the surface of red blood cells, which behaves as native nitrogen. Phenotypic expression at this locus is codominant because an individual may exhibit either one or both antigenic substances. Frequencies of the two alleles vary widely among human populations (17).

Research shows that the anti-N is sometimes seen in dialysis patients, due to cross-reactions, with the residual formaldehyde from sterilizing the equipment. This is usually irrelevant in transfusion since this variant of the antibody does not react at body temperature (18).

Following paucity of information on the occurrence and percentage distribution of Lewis B, Rh-e, N blood group antigen amongst descents of Bonny, it is therefore, necessary to carry out serological identification of Lewis B, Rh-e and Nantigens in other to access the percentage expression of these antigens are in the population. This will enable medical Scientist, Blood bank facilities within the kingdom to possibly associate their occurrence with disorders (especially haemolytic disease of the newborn, transfusion related reactions), project for the stocking of negative compatible blood for transfusion. This work is thus aimed at determining the percentage expression of Lewis B, Rh-e, N and ABO blood group antigens in descents of Bonny Kingdom Rivers State Nigeria.

Materials and Methods

Study Design and Population

This cross-sectional study involved one hundred and twenty (120) apparently healthy subjects consisting of sixty (60) females and sixty (60) males aged between 18-50 years all indigenes of Bonny Kingdom recruited by structured questionnaire for the study.

Four millilitres (4mls) of Venepuncture blood sample was obtained aseptically from the antecubital fossa of each participant with the use of vacutainer containing 0.5 mL of 1.2 mg/mL of dipotassium ethylene diamine tetra-acetic acid (EDTA) and mixed by gentle tilting for the serological determination of Lewis B, Rh-e, N and ABO blood group antigens respectively.

Materials and Methods

Determination of ABO Blood Group Using Tube method

Procedure

Red blood cells were phenotyped for A, B, AB and O antigens according to standard serologic protocol (tube method).

Determination of Lewis-B, Rh-e and N Blood Group Antigen Using Anti-le^{b}, Anti Rh-e and Anti- N Monoclonal, Lorne Laboratories Microtitre Agglutination Techniques

Phenotyping of red cells was done using Micro-titre Agglutination technique as describe by Lorne

laboratory Ltd. A 5% suspension of red blood cell was prepared using normal saline. 20ul of anti-Le^b, anti-Rh-e and anti-N antibodies were added unto separate micro-titre plate, and 20ul washed red cell was added into the micro-titre plate containing the anti-Le^b, anti Rh-e and anti-N antibodies. The sample was incubated for 15minutes with intermittent rocking and observation for agglutination every 30 seconds. If no agglutination found after 30miutes 20ul of LISS antibody was added and observed for 15-30 minutes, if no agglutination, the sample was placed in a slide under the microscope and examine microscopically for agglutination. Presence of agglutination indicates a positive result and absence of agglutination indicates negative result.

Data collected was statistically analysed by simple percentage calculation and data presented in Tables.

RESULTS

The study population consisted of a total of 120 apparently healthy Bonny indigenes, 60 males and 60 females aged between 18-50 years. 60 (50%) males and 60 (50%) females as shown in Table 1. Frequency distribution of ABO blood group amongst the Study population is shown in Table 2. 22(18.3%) [14(11.6%) males and 8(6.7%) females] typed positive for A antigens, 17 (14.2% [9(7.5%) males and 8(6.7%) females typed for B antigens, 2(1.6%) all females typed for AB antigens and 79(65.8%) 35(29.1%) male and 44(36.7%) typed for O antigens in the population. The frequency distribution of Rh-e Blood Group amongst the study population is shown in table 3 One hundred and thirteen (113) subjects from the total population (120) had Rhesus-e blood group which represent 94.17%. Out of the total population, fifty five (55) subjects who had Rh- e blood group were male while fifty eight (58) subjects were female. This represents 45.83% and 48.33% respectively. For the N Blood Group 7.5% frequency was obtained (9) subjects from the total population studied. Of the 9 persons four (4) were males while five (5) were females representing 3.3% and 4.2% respectively. Five (5) subjects from the total population had blood group Lewis B. This represents 4.16% and of this, three (3) were males while two (2) were females thus representing 2.5% and 1.7% respectively (Table 3)

Table 1: Demographic Data of study population

Gender of Subjects	Number of participants	Frequency (%)
Male	60	50
Female	60	50
Total	120	100

Table 2 Frequency Distribution of ABO Blood Group Amongst the Study Population

ABO antigens/Gender	Number of participants	A-antigen (%)	B-antigen (%)	AB- antigen (%)	O- antigen (%)
Male	60	14 (11.6%)	9 (7.5%)	0 (0%)	35 (29.1%)
Female	60	8 (6.7%)	8 (6.7%)	2 (1.6%)	44 (36.7%)
Total	120	**22 (18.3%)**	**17 (14.2%)**	**2 (1.6%)**	**79 (65.8%)**

Table 3 Frequency Distribution of Rhesus e, N, & Lewis b, Blood Groups amongst the Study Population

Subjects/ Blood groups Rh -e	Number of participants	Frequency (%)
Male	60	55 (45.8)
Female	60	58 (48.3)
Total	120	113 (94.2)
N blood Group		
Male	60	(43.3)
Female	60	5 (4.2)
Total	120	**9 (7.5)**
Lewis b		
Male	60	3 (2.5)
Female	60	2 (1.7)
Total	120	**5 (4.2)**

Discussion

This is a cross-sectional study carried out among indigenes of Bonny kingdom in Rivers State, Nigeria to assess the frequency distribution of Lewis B, Rhesus e, N and ABO blood groups amongst Bony indigenes. One hundred and twenty apparently healthy subjects consisting of sixty (60) females and sixty (60) males, age between 18 to 50 years participated in this study.

From the result obtained, it was shown that five (5) subjects, three (3) males and two (2) female (i.e 2.5% and 1.67%) respectively from the total population were positive for Lewis B blood group. This represents 4.16% of the total population. This finding is lower than the report of Christian *et al.*, (19) who in their study carried out amongst one hundred (100) Ogoni indigenes, reported a percentage distribution of 11.88% in the total population with a frequency occurrence of 12 for Lewis B (Le^b) blood group. This is also in deviant from the percentage distribution of 23 % as reported by Lorne Laboratories, (20), amongst African-Americans and also not in tandem with Reid *et al.* (21), who reported a high percentage distribution of 55% amongst Blacks. The result obtained can be due to the population size used in the study and the peculiarity of the population under study.

The result for Rh-e antigens shows that one hundred and thirteen (113) subjects from the total population had Rh-e blood group. This represents 94.2%. Out of the total population; fifty five (55) subjects were male while fifty eight (58) subjects were female representing 45.83% and 48.33% respectively. This pattern of result is in discord with a study by Christian *et al.* (8). In their study carried out amongst one hundred and one (101) Ogoni indigenes, the Rh-e blood group distribution amongst the study, revealed a percentage distribution of 25.74%. The finding in this study is also in contrast with Reid *et al.* (21) who reported a 21% Rh-e percentage distribution amongst Indians. The high prevalence reported seen in this study can be related the peculiarity of the race/population under study and also due to the high rate of blood transfusion practices, blood donation and possibly exposure from pregnancies amongst the population.

A total of nine (9) subjects from the total population had N blood group, representing 7.5% of the total population, four (4) males and five (5) females representing 3.3% and 4.17% respectively. These observations were at deviant from the frequencies in the Eastern Province of Saudi Arabia as reported by Owaidah *et al* (10) who reported the frequencies of the MNS antigens as follows: M antigen (87%), N (52%), S (59%), and s (83%) in one hundred Saudi arabia blood donors. Also finding in this research was not in tandem with the research of Hallawani *et al* (22) who found a frequency expression of 51.67% in 149 randomly samples anonymous Saudi blood donors living in Jazan Province southwestern Saudi Arabia. Research shows that the anti-N is sometimes seen in dialysis patients, due to cross-reactions with the residual formaldehyde from sterilizing the equipment although this is usually irrelevant in transfusion since this variant of the antibody does not react at body temperature (18). However, the low prevalence seen in this research could be attributed to the peculiarity of the race/population under study, the fact that subjects in this population may not have been exposed to dialysis as compared to other study population in literatures and also due to the small number of samples used in the study.

The ABO blood group system percentage expression amongst studied population revealed a total of 22 (18.3%) individuals expressed the A-antigen, 17(14.2%)

individual expressed the B-antigens, 2 (1.6%) all females expressed the AB-antigens while 79 (65.8%) of the total population expressed no A or B antigens thus representing the O-blood group. This is in agreement with a study by Erhabor *et al.*, (23). In their study, they recorded similar pattern of distribution in ABO/Rh blood group in students of Niger Delta University. Bakare *et al.* (24) recorded the similar pattern of ABO blood groups distribution in Nigerian Ethnic groups. The blood group O of the ABO system is thus the most highly expressed amongst the study population and in almost all the population and ethnic groups as revealed in literatures.

Conclusion

Rh-e phenotype occurred highest among the study participants. The proportion of the population without the expression of these antigens of the blood group tested has the potential to be alloimmunized during blood transfusion and develop antibodies, some of which can be responsible for transfusion reactions and haemolytic disease of the new born. This study has provided baseline data on the distribution of some blood group antigens in the Boony Kingdom of Rivers State in Nigeria. Based on the finding in this study, it is recommended that Rh-e grouping be carried out on pregnant mothers, blood donors and recipients before transfusion; while Lewis B and N blood groups may be subjected to expansive population testing.

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