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Research Paper

Prevalence of Lewis A, Rh-c, Mand ABO/Rh-D Antigens in Bonny Kingdom Rivers State Nigeria

Ransom Baribefii Jacob[1*]., Mueka Gift Azia[1]., Serekara Gideon Christian[1]., Legborsi Robinson-Mbato[2] andEvelyn Mgbeoma Eze[1]

[1]*Department of Medical Laboratory Science, Rivers State University, Port-Harcourt, Rivers State, Nigeria*
[2]*Department of Nursing, Rivers State University, Port-Harcourt, Rivers State, Nigeria*
Corresponding Author: Jacob, Ransom Baribefii
Department of Medical Laboratory Science, Rivers State University, Port-Harcourt, Rivers State, Nigeria

ABSTRACT
There is paucity of information on the prevalence of Lewis A, Rh-c, M and ABO/Rh-D antigens in Bonny Kingdom Rivers State Nigeria. This cross-sectional study was carried out amongst indigenes of Bonny Kingdom with the aimed of determining the prevalence of Lewis A, Rh-c, M and ABO/Rh-D antigens in Bonny Kingdom, Rivers State Nigeria. 120 apparently healthy Bonny indigenes, 60 males and 60 females aged between 18-50 years were recruited for the study. 4mls of venous blood was aseptically collected from each participant into EDTA vacutainer anticoagulant bottle from which a 5% cell suspension was prepared and used to determine the various blood groups antigens using Micro-titre Agglutination technique and Tile method respectively. Data obtained were statistically analyzed by simple percentage calculation. Results showed a total absence (0%) of blood group M antigens in the study population.112 (93.3%) participants were positive for Rh-c antigens, out of which 57(47.5%) were females and 55(45.8%) were males in the study population. 12 (10%) participant were positive for Lewis A antigen, out of which 3(2.5%) were females and 9(7.5%) were males in the study population. The ABO system showed that a total of 76 (63.3%) had O^+ blood type, 21 (17.5%) had A^+ blood type, 17 (14.2%) had B^+ blood type and 3 (2.5%) had O^- blood type with complete absence of B^- and AB^- in the study population. This study has established a complete absence (0%) of M blood antigen, 93.3% of Rh-c antigen, 10% Lewis A antigen amongst indigenes of Bonny Kingdom. Following the high frequency of Rh-c; and presence of Lewis A among the population and their association with haemolytic disease of the Newborn, haemolytic transfusion reactions and Helicobacter infection, It is recommended that these antibodies be screened for prior to transfusion, management of thrombotic disorders and Helicobacter infection.

KEYWORDS:*Prevalence, Anti-M, Anti-Rh-c,Lewis A, Bonny kingdom.*

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I. INTRODUCTION

The Kingdom of Bonny, otherwise known as Grand Bonny is a traditional statein 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 (1).

The Lewis blood group system designated by the LE (007) by the International Society of Blood Transfusion (ISBT) is located on chromosome 19p13.3 with gene name LE (FUT3). They are attached to lipids and protein in secretion having about six associated antigenssuch as Le^a, Le^b, Le^{ab}, Le^{bh}, $ALe^b$and BLe^b which are all carbohydrates found on type1 precursor chains only (2,3).

The frequency distribution of Le^ain Whites and in Blacks are 22% and 23% respectively, while the frequency distribution Le^bin Whites and in Blacks are 72% and 55 % respectively (2,4,5). The Lewis antigens are weakly expressed in cord blood, their expression in human begins at 2 years old (5).The Legene expresses a fucosyl-transferase that adds fucose in an α1 -4 linkage to the sub-terminal GlcNAc of the Type-1 chain only; galactose is already in α1 -4 linkage to the sub-terminal GlcNAc of the Type-2 chain.The Le^a allele is silent,

while the Le^b allele produces a single antigen that is found as Le^ain non-secretor [glycosphingolipid with the oligosaccharide chain attached through D-glucose] and as Le^bin secretors [glycoprotein with the oligosaccharide chain attached through N-acetyl-D galactosamine] (2,3).

In human plasma, Lewis antigens are 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. The Lewis antigens do not have their synthesis on the red blood cells, but are absorbed from plasma (4).They react at room temperature and they both activate complement. Anti-Le^ararely causeshaemolytic transfusion reaction, while anti-Le^bdoes not. Both antibodies do not cause haemolytic disease of the new-born. Anti-$Le^a$is common in pregnancy, anti-$Le^b$is not clinically significant (6).Lewis-b(Le^b) is a receptor for Helicobacter pylori (5).

The Rh blood group system is composed of forty-eight different antigens, with antigen D being the most important clinically immunogenic antigen. For clinical purposes, when testing for the Rh D antigen, the classification is by D+ (Rhesus-positive) or D– (Rhesus-negative). Most Rh-D negative individuals will automatically produce anti-D when they are transfused with blood that is Rh-D positive. The anti-D will then cause haemolysis of the recipient's red cells; and if it is haemolytic disease of the new born, it is as a result of a raised anti-D antibody in the mother, due to an initial transfusion of Rh positive blood to the negative mother or sensitization of the mother as a result of a previous pregnancy with a Rh positive baby (7).

The Rh antigens: C, c, E, and e are not so immunogenic. They only become important in patient care upon the development of the corresponding antibody. Amongst the Whites, Rh-E+ in terms of frequency of occurrence is 30% while amongst the Blacks, it is 21% (4,5). Rh-D and E antibodies are mainly IgG in nature, although there are some of them that are IgM in nature, and mostly react at 37ºC with anti-human globulin (8).

The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen will produce anti-D if they encounter the D antigen on transfused RBCs (causing a haemolytic transfusion reaction, HTR) or on fetal RBCs (causing haemolytic disease of the newborn HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be (9).

Despite the importance of the Rh antigens in blood transfusion and HDN, the physiological function of the protein can only be speculated, which may involve transporting ammonium across the RBC membrane and maintaining the integrity of the RBC membrane (10).

The Rh-c-antigen (little c) which is found in approximately 80% of the United States population, is considered the most clinically significant Rh antigen after D and is associated with severe HDN. Anti-c antibodies arise through previous exposure, such as fetomaternal haemorrhage or transfusion, and can produce acute and delayed haemolytic reactions (11). As with the D antigen, pregnant women and girls are usually sensitized to the c-antigen during an initial pregnancy, and complications occur with repeat exposure during subsequent pregnancies. Pregnancies complicated by anti-c are not extremely common; however, one may gather an idea of their incidence from the retrospective review by Hackney and colleagues (12).

Similar to the other Rh antibodies, anti-c is also primarily of the IgG type. IgM anti-c, however, has been reported, as well as other Rh IgM antibodies. As IgM antibodies are the first immunoglobulins to be produced during any humoral immune response followed by IgG, it is not unexpected that the process of sensitization and seroconversion after c-antigen exposure (and exposure to other Rh antigens) involves the same course of anti-c IgM production before anti-c IgG is formed (13). It is possible that the IgM component of the antibody does not remain very long in that phase and is difficult to capture, or it is also possible (and more likely) that the existence of the IgM component is known and therefore unlikely to garner much interest in demonstrating it even when observed (13).

Anti-M′s are naturally occurring antibodies described by Wolff and Johnsson in 1933. They have been rarely associated as cause of diseases with different degrees of severity as intrauterine deaths or haemolyticdisease of the newborn HDN (3,2). The detection of anti-M in antenatal screening is a rare finding.

The most commonly encountered antibodies from the MNS blood group are directed against the M, N, S, antigens. Anti-M antibody most often occurs as a naturally occurring saline agglutinin. It is predominantly of the immunoglobulin M (IgM) type, but few may be found as partly or wholly of immunoglobulin G (IgG) type (14). It is also observed that anti-M antibody is found in sera of patients who had no exposure to red cells. Thus, anti-M antibody is not considered to be clinically significant but when found reactive at 37°C or at AHG phase, it should be considered clinically significant. It is known that antigens of the MNS blood group system are sensitive to treatment with enzymes, such as papain and ficin, as these enzymes cleave the red cell membrane sialoglycoproteins at well-defined sites. The reactivity with anti-M antibody is abolished and thus, sensitivity of the M-antigen to the proteases helps in the identification of the antibody (15).

The frequency of M-antigen varies with population. In Caucasians, the frequency of M-antigen has been reported to be 78%, in blacks, 74% in Europeans and in African Americans the frequency was reported as 78% and 70%, respectively (16). Thakral *et al.*,2010 in their study from North India report the frequency of M-

antigen to be 75.39% (17). Makroo*et al.*,2013 reported the prevalence of M-antigen as 88.7% (18). However, there, have been no reports in the literature from the Western India.

In the study by Yasuda *et al.*, 2015, it was reported that all the 13 cases of anti-M antibody identified were biphasic in nature. Since they are reactive at 37°C, it may be considered as clinically significant and further be investigated for the potential to cause HTR and HDFN(19).

Research have associated Lewis blood group antigens as risk factor for thrombotic diseases andinfection with *Helicobacter pylori* (17); The Lewis, M, Rh blood group system is yet to be studied extensively in Rivers State, Nigeria and specifically amongst the indigenes of Bonny Kingdom. How these antigens of the Lewis, M, Rh-c together with the ABO/Rh-D blood group antigens are distributed amongst indigenes of Bonny Kingdom ethnic group is yet to be known or published in literatures accounting for the paucity of information on the prevalence of Lewis A, Rh-c, M and ABO/RhD antigens in Bonny Kingdom, Rivers State Nigeria, thus this research work was set out to synthesize knowledge on the prevalence of Lewis A, Rh-c, M and ABO/Rh-D blood group antigens amongst decent of Bonny Kingdom Rivers State Nigeria. Finding from this study will be a bank of valuable knowledge adding to the prevention and management strategies of the disorders/diseases associated with these blood types.

II. MATERIALS AND METHOD

2.1 Study Design and Population

This is across-sectional study involving one hundred and twenty (120) apparently healthy human subjects all indigenes of Bonny Kingdom Rivers State Nigeria. The participants comprised of 60 males and 60 females aged between the ages of 18-50 years recruited by the administration of questionnaires with exclusion of non-indigenes.

2.2 Sample Collection

After pre-test counselling and explanations, venous blood was drawn aseptically from the antecubital fossa of the subject with the use of vacutainer as described by Cheesebrough. Four (4.0) mL of venous blood was collected into a glass vacutainer sample bottle that contains 0.5 mL of 1.2 mg/mL of dipotassium ethylene diamine tetra-acetic acid (EDTA), it was well mixed for the serological determination of Lewis A, Rh-c, M and ABO blood group antigens respectively.

2.3 Methodology

Determination of ABO Blood Group Using tile method

Principle

The presence or absence of the A, B, AB and O antigens on human red blood cells can be determined by testing the red blood cells with the respective anti [sera, specifically Anti-A, Anti-B, Anti-AB and Anti-D. The procedure is based on the principle of agglutination of antigens with the corresponding antibodies when equal amount of the whole blood is mixed with antisera.

Procedure

For ABO and Rh-D blood group, a drop of anti-A, anti-B, anti-AB and anti-D (Atlas Medical), each is placed in the wells on the tile A, B, AB and O. A drop of red cell was added to the part labelled A, B, AB and O and anti-A, anti-B, anti-AB and anti-D was dropped in the part labelled A, B, AB and O. The mixture was mixed gently and rocked for about 30sec and observed for agglutination. Presence of agglutination indicated a positive result while absence of agglutination indicates a negative result.

Determination of Lewis-a, Rh c and M Blood Group Using Anti-Le^a, Anti-Rh-c and Anti- M 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. 20µl of anti-Le^a, anti-Rh-c and anti-M antibodies were added unto separate micro-titre plate, and 20µlwashed red cell was added into the micro-titre plate containing the anti-Le^a, anti Rh-c and anti-M antibodies. The sample was incubated for 15minutes with intermittent rocking and observation for agglutination every 30 seconds. If no agglutination found after 30minutes,20µl 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.

2.4 Data Analysis

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

III. RESULTS

3.1 Demographic data of participants

The study population consisted of a total of 120 apparently healthy Bonny indigenes, 60 males and 60 females aged between 18-50 years. Out of the 60 (50%) males, those within the ages of 18-30 were 54 (45%) and those within the ages of 31-50years were 6 (5%) while of the 60 (50%) females, those within the ages 18-30 were 51 (42.5%) and those within the ages of 31-50years were 9 (7.5%) as shown in Table 3.1

Table 3.1Demographic Data of Participants in the Study Population

Average Age (yrs)	Gender	Frequency	Percentage (%)
18-30	Male	54	45%
31-50	Male	6	5%
	Total	**60**	**50%**
18-30	Female	51	42.5%
31-50	Female	9	7.5%
	Total	**60**	**50%**
Grand Total		120	100%

3.2 Frequency Distribution of Le^{a}Blood Group Antigen amongst the Study Population

Table 3.2 shows a frequency distribution of Le^{a}antigen amongst the study population. A total of 12 (10%) of the population were positive for Le^{a}blood group antigen 3(2.5%) females and 9(7.5%) males.

Table 3.2Frequency Distribution of Le^{a}Blood Group antigen amongst the Study Population

Subjects/Gender	Total Population	Frequency	Percentage (%)
Male	60	9	7.5
Female	60	3	2.5
Total	120	12	10

3.3 Frequency distribution of Rh-c blood group antigen amongst the study population

Table 3.3 Shows a frequency distribution of Rh-c blood group antigen amongst study population. A total of 112 (93.3%) of the population were positive for Rh-c blood group antigen 57(47.5%) females and 55(45.8%) males.

Table 3.3Frequency Distribution of Rh-c Blood Group amongst the Study Population

Subjects/Gender	Total Population	Frequency	Percentage (%)
Male	60	57	47.5
Female	60	55	45.8
Total	120	112	93.3%

3.4 Frequency distribution of M blood group antigen amongst the study population.

Table 3.4 shows a frequency distribution of the M blood group amongst the study population. No positive test for M blood group antigen 0(0%) females and 0(0%) males was found in the study population.

Table 3.4Frequency Distribution of M Blood Group amongst the Study Population

Subjects/Gender	Total Population	Frequency	Percentage (%)
Male	60	0	0
Female	60	0	0
Total	120	0	0%

3.5 Frequency Distribution of ABO/Rh-D Blood Group amongst the Study Population.

Table 3.5 shows a frequency distribution of ABO/Rh-D blood group amongst the study population. A total of 21 (17.5%) of the population were of blood group A+ 8(6.7%) females and 13(10.8%) males. A total of 1 (0.8%) of the population were of blood group A- 0(0%) females and 1(0.8%) males. A total of 17 (14.2%) of the population were of blood group B+ 8(6.7%) females and 9(7.5%) males. None of the population was positive blood group B- 0(0%) females and 0(0%) males. A total of 2 (1.7%) of the population were of blood group AB+ 2(1.7%) females and 0(0%) males. None of the population was positive for blood group AB- 0(0%) females and 0(0%) males. A total of 76 (63.3%) of the population were of blood group 0+ 37(30.8%) females and 39(32.5%) males. A total of 3 (2.5%) of the population were of blood group O- 3(2.5%) females and 0(0%) males.

Table 3.5Frequency Distribution of ABO/Rh-D Blood Group amongst the Study Population

Blood Group	Gender	Frequency	Percentage (%)
A+	Male	13	10.8
	Female	8	6.7
	Total	**21**	**17.5**
A-	Male	1	0.8
	Female	0	0
	Total	**1**	**0.8**
B+	Male	9	7.5
	Female	8	6.7
	Total	**17**	**14.2**
B-	Male	0	0
	Female	0	0
	Total	**0**	**0**
AB+	Male	0	0
	Female	2	1.7
	Total	**2**	**1.7**
AB-	Male	0	0
	Female	0	0
	Total	**0**	**0**
O+	Male	37	30.8
	Female	39	32.5
	Total	**76**	**63.3**
O-	Male	0	0
	Female	3	2.5
		3	**2.5**
	Total	**120**	**100**

IV. DISCUSSION

The Bonny Kingdom is not a major tribe in Nigeria, not also related to any other tribe in Nigeria.Bonny Kingdom served as the contact point between some Portuguese and locals during the 15th century when it became one of the most popular slave trade centres (1).

The research work was set out to determine the prevalence of rare blood group antigens such as the Lewis, M, Rh-c in indigenes of Bonny Kingdom Rivers State Nigeria. The finding from this study revealed a total absence of M Blood group in the study Population with a frequency of zero percentage (0%). This result shows a fair agreement with the research carried out by Rashmi *et al*., 2008, who did not find the M blood antigens to be fairly common and detected only two cases of anti-M in a period of 3years in the study population (20). Also our findings are not in tandem with the frequency of anti-M antibody in a study done by Shah *et al.,* 2016 who found anti-M antibodies to be 13.98% (13/93) in multigravida females aged between 11months to 85years in their study population (21), Petras *et al*., 2012 in their study, reported the frequency of anti-M antibody as 2.9% (197/6769) (22) and Tormey *et al*. 2008 who reported a prevalence of 3.45% (18/521) in a population of male military veterans (23). Furthermore,Thakral *et al.,* 2010 in their study from North India report the frequency of M-antigen to be 75.39% (17). Makroo*et al.,* 2013 reported the prevalence of M-antigen amongst population of blood donors in Indian to 88.7% (18).

The absence of M blood amongst the descents of Bonny Kingdom might be as a result of the fact that M blood group is not commonly found in blacks. Anti-M′s are naturally occurring antibodies described by Wolff and Johnsson in 1933. They have been rarely associated as cause of diseases with different degrees of severity as intrauterine deaths or haemolytic disease of the newborn haemolytic disease of the newborn (HDN). Also, the detection of anti-M in antenatal screening is a rare finding thus, the total absence of the M blood group amongst the descents of Bonny might be an indication of a low degree of intrauterine deaths or haemolytic disease of the newborn HDN due to anti-M among its indigenes.

This study has also revealed the presence of Le^{a}amongst the Bonny Descents. A total of 12 (10%) of the study population were positive for Le^{a}blood group antigen, 3(2.5%) females and 9(7.5%) males. This finding in terms of percentage distribution is lower than the finding reported by Lorne Laboratories (24), where they reported 23% amongst Afro-Americans.Findings in this study are in deviant from the research of Reid *et al.,* 2012 who reported a percentage distribution of 23% (25). The low findings might be as a result of the fact that the Lewis antigen is weakly expressed in humans since their expression in human begins at 2 years old (5). Also, finding in this study is lower than that of Christian et al., 2020 (26) who had earlier reported a prevalence of 17.8% amongst the Ogoni ethnic group of Rivers State, Nigeria. In human plasma, since the Lewis antigens are attached to erythrocytes, platelets and lymphocytes that are circulating and do not have their synthesis on the red blood cells, but are absorbed from plasma (4), this could also be the reason for its poor prevalence in indigenes of Bonny Kingdom.

The study further revealed a prevalence of 93.3% in the total population with a frequency occurrence of 112 out of 120 subjects for the Rh-c blood group antigen. This finding is similar to that of Christian et al., 2021

(27)that reported a 100% prevalence for Rh-c amongst pregnant women in River State, but not in tandem with the percentage distribution of 23 % as reported by Lorne Laboratories, (24), amongst Afro-Americans, and also in discord with Reid *et al.,* 2012 who had earlier reported a lower percentage distribution of 55% amongst Blacks (25). The high frequency of Rh-c in the population might be as a result of the fact that since the Rh-c-antigen (little c) are not assay for during pretransfusion screening in Bonny Indigenes, the rate of exposure to these antigens must have occurred during delivery, blood transfusion or post-partum haemorrhage thus accounting for the high prevalence rate of 93% in the study population. The Rh-c is considered the most clinically significant Rh antigen after D and is associated with severe haemolytic disease of the new born (HDN). Anti-c antibodies arise through previous exposure, such as feto-maternal haemorrhage or transfusion, and can produce acute and delayed haemolytic reactions (11). As with the D antigen, pregnant women and girls are usually sensitized to the c-antigen during an initial pregnancy, and complications occur with repeat exposure during subsequent pregnancies (12).

The ABO blood group amongst the study population (descents of Bonny Kingdom), Rivers State, revealed that blood group O+ have the highest occurrence with a total of 76 (63.3%) of which 37(30.8%) females and 39(32.5%) males, followed by blood group A+ with a total of 21 (17.5%) of which 8(6.7%) females and 13(10.8%) males and blood group B+ a total of 17 (14.2%) of the population of which 8(6.7%) females and 9(7.5%) males. The highest frequency seen in blood group O+ is as a result of the presence of Rh-D which is the most occurring blood group antigen. There was a complete absence of the B-(Negative) and AB- (negative) in the study population indicating that the antigens are rare and less occurring.

V. CONCLUSION

The results obtained from this study showed a zero-prevalence rate for blood group antigen M;andB-, AB- antigensof the ABO blood group system indicating a complete absence of these blood group antigens in the study population. However, a prevalence rate of93.3% for Rh-c and 10% for Lewis A blood group antigen was seen amongst the study population of Bonny Kingdom, Rivers State Nigeria.

VI. RECOMMENDATIONS

Following the high prevalence rate of Rh-c antigens amongst study population, further study should be carried out amongst pregnant women of bonny kingdom to ascertain the actual cause of transfusion reaction and find out their role in immunization.

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