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DISTRIBUTION OF HAEMOGLOBIN GENOTYPE, ABO AND RHESUS (D) BLOOD GROUPS AMONG PREGNANT WOMEN IN NORTH CENTRAL NIGERIA

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ABSRACT

Background: 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 Availability of data on the distribution of these genetic markers in central Nigeria would help in the planning and management of patients with related disorders. **Aim:** This study is designed to determine the distribution of haemoglobin genotype, ABO and Rhesus blood groups among pregnant women that are crucial information for the control of hereditary erythrocytic disorders. **Method:** The blood samples of 222 pregnant women attending the Antenatal clinic in Plateau State Specialist Hospital, Jos between April to June, 2013 were collected and their haemoglobin genotypes were determined using cellulose acetate electrophoresis at alkaline (Tris EDTA) buffer pH 8.6; ABO and Rhesus blood groups were determined by a standard tube method. **Results:** A total of 222 subjects were screened for haemoglobin genotype, ABO and Rhesus (D) blood groups. The distribution of haemoglobin genotypes were 164 (73.9%) for HbAA and 58 (26.1%) for HbAS. The frequencies of ABO blood groups among the study population were 41 (18.5%), 50 (22.5%), 12 (5.4%) and 119 (52.6%) for blood group A, B, AB, and O respectively. Also, the distribution of Rhesus (D) positive and Rhesus (D) negative were reported as 203 (91.4%) and 19 (8.6%) each. There was no association between ABO, Rhesus (D) and Hb genotypes observed. Data revealed that Rhesus (D) positive pregnant women were statistically higher in number compared to the Rhesus (D) negative women (p=0.000). **Conclusion:** The determination of the distribution of haemoglobin genotypes, ABO and Rhesus blood groups with a frequent review is essential in patients' management and control policy.

KEYWORDS: Haemoglobin, erythrocytic, haemoglobinopathies.

INTRODUCTION

Nigeria is one of the African countries that are vulnerable to hereditary erythrocytic disorders such as; sickle cell disease, haemolytic disease of new born and haemoglobinopathies (Worlledge, Ogiemudia et al. 1974, Akhigbe, Ige et al. 2009). Haemoglobin genotype, ABO and Rhesus blood groups are inherited blood markers. The distribution of haemoglobin genotype, ABO and Rhesus blood groups vary from one population to another.

Haemoglobin is very essential in the transportation of oxygen within the body (Lukin and Ho 2004, Safo, Ahmed et al. 2011). It has the property of combining reversible with oxygen. Oxygen is rapidly taken up by the erythrocyte in the lungs, during the few milliseconds the cells take to pass through the pulmonary microcirculation. There is a rapid saturation of haemoglobin molecule with oxygen, to form oxy-haemoglobin due to the enhancing interaction of the four haem group in an environment with high oxygen tension (Scott 1999).This process is reversed when the erythrocyte passes through tissue with low oxygen tension, the oxygen is then released (Carreau, Hafny-Rahbi et al. 2011). Haemoglobin also plays a part in the transport of carbon dioxide to the lungs (Scott 1999). In man, six main types of haemoglobins are present and they correspond with different stages of human development. The normal haemoglobins are as follows based on stages of human development; from embryonic to foetal to adult types: Hb Gower-1 ($\zeta 2\varepsilon 2$), Hb Gower-2 ($\alpha 2\varepsilon 2$), Hb Portland -1 ($\zeta 2\gamma 2$), Hb Portland -2 ($\zeta 2\beta 2$), Foetal Hb ($\alpha 2\gamma 2$), HbA_2 ($\alpha 2\delta 2$) and HbA ($\alpha 2\beta 2$) (Fantoni, Farace et al. 1981, Huisman 1993, Manning, Russell et al. 2007). Any form of haemoglobin that differs from the above haemoglobin types is variant haemoglobin. Examples are haemoglobin S and haemoglobin C (Soranzo, Sanna et al. 2010).

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 (Landsteiner 1900). 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 (Dean 2005). Individuals are classified into four major ABO blood groups namely; A, B, AB and O depending on the antigen present on their RBC surface (Sembulingam and Sembulingam 2012, Mitra, Mishra et al. 2014).

Rhesus blood system was discovered following demonstration of an antibody in a group O mother who delivered a dead foetus. Levine and Stetson in 1939 found that antibody present in the serum is due to stimulation of antigenic factors present in the foetus (Levine and Stetson 1939). The human red blood cells that carry antigen D are referred to as Rhesus positive (Rh^+) while those without antigen D are Rhesus negative (Rh^-). Antigen D is the most immunogenic of the Rhesus antigens, it is also a major cause of haemolytic disease of the newborn (Egesie, Egesie et al. 2008).

MATERIALS AND METHODS

STUDY AREA/POPULATION

The study was carried out in Jos metropolis, Plateau state, Nigeria. The study population comprised of 222 pregnant women attending Antenatal clinic in Plateau State Specialist Hospital, Jos. Nigeria. Study samples were processed in the Haematology/Blood Transfusion Unit of Plateau Specialist Hospital, Jos.

INCLUSION AND EXCLUSION CRITERIA

Consented pregnant women that attended antenatal clinic in Plateau State Specialist Hospital, Jos were included in the study, while pregnant women whose consent was not obtained and pregnant women attending antenatal clinic in other tertiary hospitals in Plateau state were excluded from the study.

ETHICAL CLEARANCE

This study was approved by the plateau state specialist hospital ethical committee and the informed consent was obtained from all the study participants.

SAMPLE COLLECTION

Disposable needles and syringes were used to collect 2ml of blood from each participant by venipuncture and transferred into appropriately labelled ethylenediamine tetraacetic acid (EDTA) and plain blood specimen bottles.

BLOOD GENOTYPING

Haemoglobin Genotyping of the participants were determined using cellulose acetate paper electrophoresis technique. Each sample was diluted using haemolysate to get haemoglobin concentration of approximately 10g/100ml. With the aid of an applicator, haemolysed blood sample was placed on the cellulose acetate paper. Electrophoresis in Tris buffer solution was for 15-20 minutes at 350v. Haemolysed blood samples of known HbAS and AC were ran as controls.

ABO AND RHESUS (D) BLOOD GROUPING

The ABO and Rhesus (D) blood typing were carried out using standard tube method. Serum was taken for the serum grouping; red blood cells were extracted from the clotted blood, washed in normal saline and diluted to 2% saline for cells grouping. The test for ABO grouping was incubated at room temperature (22^oc) for 2hrs.The test for Rh (D) grouping was incubated at 37^oc for 2hrs. All negative reactions were confirmed microscopically.

STATISTICAL ANALYSIS

Data analysis was carried out using statistical package for social sciences (SPSS) version 17.0 software. Results of the analysis were presented in numbers and percentages in the form of frequency tables. Statistically significant was tested using Chi-square statistic. $P < 0.05$ was considered statistically significant.

RESULTS

1: The distribution of Haemoglobin Genotype among study population

The distribution of Haemoglobin Genotype among 222 samples collected and analysed in the study population are 164(73.9%) were Haemoglobin genotype AA (HbAA) and 58(26.1%) were Haemoglobin genotype AS (HbAS). Data shows that the distribution of normal HbAA among study population is significantly higher compared to those with HbAS variant (P= 0.000) as shown in Table 1 below:

Table 1: Distribution of Genotype among Pregnant Women Attending Antenatal Clinic in Plateau State Specialist Hospital.

Genotype	Number	Percentage (%)	P
AA	164	73.9	0.000
AS	58	26.1	
Total	222	100.0	

KEY: AA and AS are Haemoglobin Genotypes.

2: The distribution of ABO Blood Groups among study population

The distribution of ABO blood group among 222 samples collected and analysed of study population are: Blood group O were 119(53.6%), Blood group B were 50(22.5%), Blood group A were 41(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 2: Distribution of Blood Group among Pregnant Women Attending Antenatal Clinic in Plateau State Specialist Hospital.

Blood group	Number	Percentage (%)	P
A	41	18.5	0.000
B	50	22.5	
AB	12	5.4	
O	119	53.6	
Total	222	100.0	

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

3: THE DISTRIBUTION OF RHESUS (D) BLOOD GROUPS AMONG STUDY POPULATION

The distribution of Rhesus (D) blood groups among 222 samples collected and analyzed of study population are: 203((91.4%) were Rhesus positive and 19(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 3 below:

Table 3: Distribution of Rhesus (D) Blood Groups among Pregnant Women Attending Antenatal Clinic in Plateau State Specialist Hospital.

Rhesus status	Number	Percentage (%)	P
Positive	203	91.4	0.000
Negative	19	8.6	
Total	222	100.0	

4: THE RELATIONSHIP BETWEEN ABO AND RHESUS (D) BLOOD GROUPS

Out of 222 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 4 below:

Table 4: Relationship between ABO and Rhesus (D) Blood Groups.

Parameter	Blood Group				
	A	B	AB	O	P
Rhesus					
Positive	41(100.0)	46(92.0)	11(91.7)	105(88.2)	0.143
Negative	0(0.0)	4(8.0)	1(8.3)	14(11.8)	
Total	41(100.0)	50(100.0)	12(100.0)	119(100.0)	

5: THE RELATIONSHIP BETWEEN ABO BLOOD GROUPS AND HAEMOGLOBIN GENOTYPES

Out of 222 samples analyzed; Blood group O had the highest HbAA and blood group AB had the lowest HbAA. Blood group O had the highest HbAS and blood group AB had the lowest HbAS. The p value (0.718) shows that there is no statistically significant difference in the relationship between ABO blood groups and Haemoglobin genotypes among the study population. This is shown in Table 5 below:

Table 5: Relationship between ABO Blood Groups and Haemoglobin Genotypes.

Parameter	Blood Group				
	A	B	AB	O	P
Genotype					
AA	29(70.7)	40(80.0)	9(75.0)	86(72.3)	0.718
AS	12(29.3)	10(20.0)	3(25.0)	33(27.7)	
Total	41(100.0)	50(100.0)	12(100.0)	119(100.0)	

6: THE RELATIONSHIP BETWEEN HAEMOGLOBIN GENOTYPES AND RHESUS (D) BLOOD GROUPS

Out of 222 samples analyzed, Hb genotype AA had the highest proportion of both Rhesus positive and Rhesus negative among the study population. The p value (0.283) shows that there is no statistically significant difference in relationship between haemoglobin genotype and Rhesus (D) blood groups among the study population. This is shown in Table 6 below:

TABLE 6: Relationship between Haemoglobin Genotypes and Rhesus (D) Blood Groups.

Genotype	Rhesus		
	Positive	Negative	P
AA	148(90.2)	16(9.8)	0.283
AS	55(94.8)	3(5.2)	
Total	203(91.4)	19(8.6)	

DISCUSSION

In this study, the distribution of HbAA and HbAS was 164(73.9%) and 58(26.1%) respectively among the study population. HbAS was the only Hb variant identified in the study. The HbSS, HbSC and HbAC were not identified among the study subjects. There is statistically

significant difference of p value (p>0.005) between the distribution of HbAA and HbAS among the participants.

The distribution of HbAA ranges from 55-75% while sickle cell trait (HbAS) is 20-30% in Nigeria (Adeyemo and Soboyejo 2006, Egesie, Egesie et al. 2008). The distribution of HbAA in this study is similar to the 77.7% reported by Damulak and Colleagues in Jos Nigeria(Damulak, Bolorunduro et al. 2013). It is also similar to that reported in the Bonny area of Rivers State and Uyo Nigeria, where HbAA accounted for 73% and 78% respectively(Adeyemo and Soboyejo 2006, Egesie, Egesie et al. 2008).The distribution of HbAS is 26.1% in this study correlate with some previous studies (Uzoegwu and Onwurah 2003, Damulak, Bolorunduro et al. 2013)(which are 26.94% and 21.88% respectively.

The distribution of ABO blood groups varies from one population to another. This study shows that 119(53.6%) were blood group O, 50(22.5%) were blood group B, 41(18.5%) were blood group A and 12(5.4%) were blood group AB. The relative frequencies of ABO blood group system in this study was in order O>B>A>AB which is not in agreement with previous studies where O>A>B>AB (Uzoegwu and Onwurah 2003, Epidi, Nwani et al. 2008).The blood group B with 22.5% is higher than blood group A with 18.5% among the study population. This study affirmed that blood group O was predominant among the ABO blood groups and blood group AB was the least.

The distribution of Rhesus D positive antigen in this study was 203(91.4%) while Rhesus D Negative had 19(8.6%). The findings of this study did not concur with the previous studies: 96.7% Rhesus positive and 3.7% Rhesus Negative (Ukaejiofor, Okonkwo et al. 1996), 96.77% Rhesus positive and 2.73% Rhesus Negative (Jeremiah 2006). There is slightly high distribution of Rhesus D Negative antigen observed in this study. This indicates the possibility of Rhesus D alloimmunization among the study population through incompatible pregnancy or transfusion; Thereby increases the incidence of haemolytic disease of newborn due toRhesus incompatibility.

This study shows that, there is no association between haemoglobin genotype, ABO and Rhesus blood groups occurrences among the study population.

CONCLUSION

The study shows that the distribution of haemoglobin variants AS concurred with report from most previous studies. ABO blood groups are not in agreement with the report from previous studies. In the study, the distribution of Rhesus blood group indicated high percentage with statistically significant differences. The study did not indicate relationship between; ABO and Rhesus (D) blood groups, ABO Blood groups and Haemoglobin genotype; and Haemoglobin genotype and Rhesus (D) blood groups. This study justifies the need for occasional review of haemoglobin genotype, ABO and Rhesus (D) blood groups distribution documented at antenatal care delivering centres. The information will provide a good platform for making policy and necessary action to intensify awareness in order to abate complication associated with hereditary erythrocytic disorder.

RECOMMENDATION

It is recommended that; Rhesus typing should be carried out in a wider scale as the study indicated high Rhesus (D) negative percentage, Annual chart describing the haemoglobin genotype and Rhesus typing of pregnant women should be documented at antenatal care delivering centres. This information will provide a good platform for policy making towards monitoring complication; such as sickle cell diseases and haemolytic disease of newborn associated with haemoglobin variants and rhesus negative respectively.

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