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ORIGINAL ARTICLE

Haemolysin test, ABO and Rhesus Blood grouping in Relation to occurrence of stillbirth, Miscarriage and transfusion status among Pregnant Women in University of Calabar, Nigeria

[1] Naomi A. Ernest, [2]Dorathy C. Okpokam, [2]Patience A. Akpan, [3]Henshaw Okoroiwu, and [2]Anthony O. Emeribe

[1]Department of Haematology Pathology, University of Calabar Teaching Hospital, Calabar, CRS, Nigeria.

[2]Department of Haematology and Blood Transfusion Science, University of Calabar, Calabar, CRS, Nigeria

[3]Department of Medical Laboratory Science, Arthur Jarvis University, Akpabuyo, CRS, Nigeria.

Corresponding Author

Dr. Dorathy C. Okpokam
Department of Haematology and Blood Transfusion Science
Faculty of Medical Laboratory Science
University of Calabar
Calabar - Nigeria

+2348068896860, +2348023552406
Emails: oghalove@gmail.com;

Abstract

Introduction: Human red blood cells contain on their surface a series of glycoproteins and glycolipids which constitute the blood group antigens, which are also related to many clinical problems associated with transfusion reactions

Aim of study: This study is aimed at providing information on the ABO and Rhesus Blood grouping in Relation to occurrence of stillbirth, Miscarriage and transfusion status among pregnant women attending Antenatal Clinic in University of Calabar Teaching Hospital, Calabar Nigeria.

Materials and Methods: A descriptive cross-sectional study comprising of 400 pregnant women, aged 16 – 45years and who gave their informed consent was used. ABO and Rhesus blood groups were analysed using commercially prepared reagent. Haemolysin test and antibody screening were performed using standard cells.

Results: Most of the pregnant women were aged 16 – 36 years and no underweight pregnant women were recorded in this study. The prevalence of ABO blood group among the pregnant women showed the decreasing order of O>A>B>AB. It was observed that blood group O appeared about 3 times the prevalent of each group. The prevalence of Rhesus positive and Negative was 95% and 5% respectively. 5.5% of stillbirth was recorded while miscarriage had a prevalence of 17%. Approximately 5% of the pregnant women received blood transfusion. Rhesus blood group was found to be associated with still-birth (P=0.004).

Conclusion: ABO blood group distribution was in the order O>A>B>AB 63%, 20%, 14% and 3.0%. On the other hand, the study shows the prevalence of Rhesus D' positive and Rhesus D' negative to be 95% and 5% respectively. Blood group 'O' and rhesus positive blood group were predominant in stillbirth, miscarriages and transfusion status. It is recommended that the use of group O blood free from haemolysin α and β should only be transfused to pregnant women because of risk to the foetus.

okpokamdora@unical.edu.ng

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Introduction

The discovery of the ABO blood group, over 100 years ago, by Landsteiner have caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood (1). After ABO blood group system, the second most significant blood group system is Rhesus system as it describes how the mother of a still born foetus suffered a severe haemolytic reaction when transfused with her husband blood .The mother who obviously lacked some new antigen must have been immunised by her foetus that possessed this antigen. (2).

Human red blood cells contain on their surface a series of glycoproteins and glycolipids which constitute the blood group antigens, which are also related to many clinical problems associated with transfusion reactions. The development of these antigens is genetically controlled and they appear early in foetal life and remain unchanged until death (3). In addition to the ABO and Rhesus (Rh) blood group antigens, there are several other blood group antigens located on the red cell surface. Transfusion recipients who lack these antigens on their red cell surface may develop antibodies when transfused with blood containing the said antigens (4).

Some blood groups are referred to as universal donors like blood group 'O', while 'AB' are said to be Universal recipient, as a result of scarcity of blood, Universal donor could sometimes be given to other blood groups where they are compatible, while Universal recipient can receive from any other group (5). It has been observed that this kind of transfusion, most at times though compatible when tested invitro could result to transfusion reaction invivo considering the fact that 'O' individual may not be neutralized, when transfused to other blood groups other than O. This group of 'O' are known as dangerous group 'O' (6).

The clinically important antibodies that are produced against red cell antigens can cause haemolytic transfusion reaction and reduce the life span of transfused erythrocytes (7). In adults with a normal immune system, these antibodies are present when the corresponding antigens are absent on the red cell, all antibodies to red cell antigens other than naturally occurring, Anti-A and Anti-B are considered to be "unexpected" mostly directed toward non-ABO system, antigens absent on the red cells or autoantibodies directed toward self-antigen (8).

Blood group antigens are inherited stable characteristics which have proven to be useful in transfusion medicine, prevention and management of haemolytic transfusion reaction and haemolytic disease in newborn infants as well as resolving cases of doubtful parentage. Cross River State, Nigeria is a vast territory with many ethnic groups (9); the distribution of rare blood groups may display unique features because of relative geographical isolation, inter-ethnic marriages and historical migration. Under ideal situations, the allele frequencies of a

given population would remain stable across generations.

Materials and Methods

Study design

The study took a prospective descriptive cross sectional approach, which was carried out in the blood group serology unit of the University of Calabar Teaching Hospital Calabar. The hospital is a tertiary health facility rendering quality health services to the people of Cross River State and neighboring States.

Study area

The selected area for this study comprised Cross River and Akwa Ibom State whose residents sought for services of the University of Calabar Teaching Hospital located along Eastern highway in Calabar Municipality within Calabar metropolitan city in Cross River State.

Selection of subjects

Four hundred (400) pregnant women attending the antenatal clinic at University of Calabar Teaching Hospital were studied. This was over a period of six (6) months (September 2020 – February, 2021), and they were aged 16-45years in their first, second or third trimester. The following socio-demographic characteristics were extracted from the patients record; parity, history of gravidae outcome and transfusion history.

Inclusion criteria

Consenting pregnant women confirmed by Consultant Obstetricians attending antenatal clinic at the University of Calabar Teaching Hospital aged 16-45 years and resident in Calabar metropolis were recruited for the study.

Exclusion criteria

Women who did not meet the eligibility criteria; non-pregnant women, non-consenting pregnant women were excluded from the study.

Collection and testing of samples

Samples were collected from the pregnant women who came for antenatal clinic at UCTH. A total of three (3) millilitres of blood was collected aseptically through vene- puncture and was dispensed into a sterile screw cap plain bottle and allowed to clot. Within one hour at room temperature, the sample was centrifuged at 3,000 rpm for five minutes and the serum was separated using a clean Pasteur pipette into a clean plain tube with screw cap for haemolysin test and antibody screening. The red cells were grouped/ typed for ABO and Rhesus.

Determination of ABO, Rhesus blood group, haemolysin and antibody screening tests

ABO blood grouping was done using anti-A, Anti-B and Anti-AB bought from Biotech (Ipswich UK).

1. **Standard Technique (Tube Method) ABO Grouping (10).**

Principle**:** When red cells are mixed with antisera A, B & AB. The red cells react specifically with the antisera to which it has corresponding antigen in an observable manner (Agglutination).
This bloodgroups are determined by the presence or absence of agglutination.

Procedure for standard tube grouping

i. Blood samples were washed in large volume of 0.85% normal saline 4 times
ii. 5% cell suspension was made
iii. The test tubes were labeled accordingly and placed in the test tube rack
iv. 1 volume of the antisera was added accordingly
v. 1 volume of 5% washed cell suspension was added to each test tube respectively.
vi. The suspension was mixed and allowed

to stand for 2hours at room temperature.
vii. It was checked macroscopically for agglutination and results recorded.
viii. Negative results were confirmed microscopically using X10 objective.

Test samples were run alongside with known controls (A2 cells B cells and O cells).

2. Rhesus grouping

Rhesus grouping was done using Anti-D monoclonal reagent bought from Biotec. (10).
Principle: The reagents will cause direct agglutination (clumping) of test red blood cells that carry the corresponding Rhesus Antigen.
Procedure:

i. All the reagents were brought to attain room temperature.
ii. The cells were washed in 0.85% normal saline and 5% suspension of the washed cells was made.
iii. One drop of Anti D was added into the labeled khan tubes and equal volume of the 5% washed red cells was added respectively.
iv. Suspension was mixed and incubated at room temperature for 2hours.
v. Observed for agglutination macroscopically and microscopically.

All Negative results were confirmed using indirect agglutination test technique with 2% bovine albumin and anti-human globulin (AHG) test at 37^0C

vi. After spinning for 20 second at 1000rpm the cell was gently re-suspended and observed macroscopically and microscopically for agglutination.

Rhesus Negative and positive controls was included in all procedure.

3. **Haemolysin test (10)**

i. All the tubes were arranged in the test tubes racks.
ii. All the reagents were brought to attain room temperature
iii. Cells was washed in 0.85% Normal saline and 5% suspension of the washed cells was made.
iv. One drop of group O serum was added to the test tubes.
v. To the first test tube equal volume of 5% suspension of A cells and to the second test tube equal volume of 5% suspension of B cells was added and incubated for 2 hours at 37^0C
vi. It was observed for haemolysis.

Interpretation of Result

If there is no haemolysis, all the red cells will be clumped at the bottom of the test tube and the supernatant serum is clear.

If there is haemolysis, the serum will be pink-red and there will be few or no cells at the bottom of the test tube.

Statistical analysis

The data collected was recorded on an excel spread sheet and later subjected to analysis using statistical software SPPS version 20.0. Statistical analysis included frequency, percentage and chi-squared tests. Differences were considered significant at $p < 0.005$.

Results

Table 1 shows the socio- demographic characteristics of the pregnant women, the majority of the study subjects were in the aged group 16 – 25years 178 (44.5%), 26 – 35years 174(43.5%), 36 -45years 48(12.0%), >46years 2(0.5%). Table 6 also showed the study subjects based on parity, the study population were distributed as primegravidae (n=250), Multigravidae (n=140) and grand Multigravidae (n=10). The percentages were observed to be 62.5%, 35% and 2.5% respectively. Those that had still birth (n=22),

miscarriage (n=68) and transfusion status (n=20) percentages were 5.5%, 17% and 5% respectively.

Figure 1 shows the prevalence of ABO Blood Grouping among the participants in which majority of the participants 252 (63%) had blood group O, followed by the blood group A 80 (20%). Blood group B had 56 (14%) while the remaining 12 (3%) had blood group AB.

Figure 2 shows prevalence of Rhesus positive and Rhesus negative antigens of pregnant women in UCTH, 380(95.0%) were positive while 20(5.0%) were negative.

Figure 3 shows the distribution of haemolysin in blood group O pregnant women in UCTH. Pregnant women with haemolysin α & β had a frequency of 85(33.7%), those with haemolysin α had a frequency of 62(24.6%) and haemolysin β had 23(9.1%).

Table 2 shows the association between occurrence of stillbirth, miscarriage, transfusion and their ABO blood group. For stillbirth, blood group A had 4(18.2%), blood group B had 4(18.2%), and AB had 0 (0.0%) while blood group O had 14(63.6%). There was no significant statistical association (X^2 =1.709, p > 0.05) between stillbirth and ABO blood groups

For miscarriage, blood group A had 8(11.8%), blood group B had 10(14.7%), blood group AB had 2(2.9%) while blood group O 48(70.6%). There was no significant statistical association (X^2 =3.557, p > 0.05) between miscarriage and ABO blood group.

For transfusion status blood group A had 4(20.0%), blood group B had 4(20.0%), blood group AB had 0(0.0%) while blood group O 12(60.0%). There was no significant statistical association (X^2 =1.203, p > 0.05) between transfusion status and ABO blood group.

Table 3 shows occurrence of stillbirth, miscarriage, transfusion status and their Rhesus blood group. For stillbirth Rhesus positive had 18 (81.8%) while Rhesus negative had 4 (18.2%), there was a significant statistical association (x^2=8.25, P>0.05) between stillbirth and rhesus blood group.

For miscarriage rhesus positive had 64(94.1%) while rhesus negative had 4 (5.9%) there was no significant association (x^2=0.13,P<0.05) between miscarriage and rhesus blood group.

Transfusion status had a rhesus positive of 18(90.0%) while rhesus negative 2(10%) there was no significant association (x^2=1.11, P > 0.05) between transfusion status and rhesus blood group.

Table 1: Socio-demographic characteristics of the participants

DEMOGRAPHIC DATA	**FREQUENCY**	**PERCENTAGE**
AGE GROUP		
16-25	178	44.5
26-35	172	43.0
36-45	48	12.0
>46	2	0.5
PARITY		
Primegravidae	250	62.5%
Multigravidae	140	35%

Grandmultigravidae	10	2.5%
STILLBIRTH		
YES	22	5.5
NO	378	94.5
MISCARRIAGE		
YES	68	17.0
NO	332	83.0
NUMBER OF MISCARIAGES		
NIL	326	81.50
1	60	15.00
2	12	3.00
≥3	2	0.50
TRANSFUSION STATUS		
YES	20	5.00
NO	380	95.00
NUMBER OF TIMES TRANSFUSED		
1	20	5.00
NONE	380	95.00

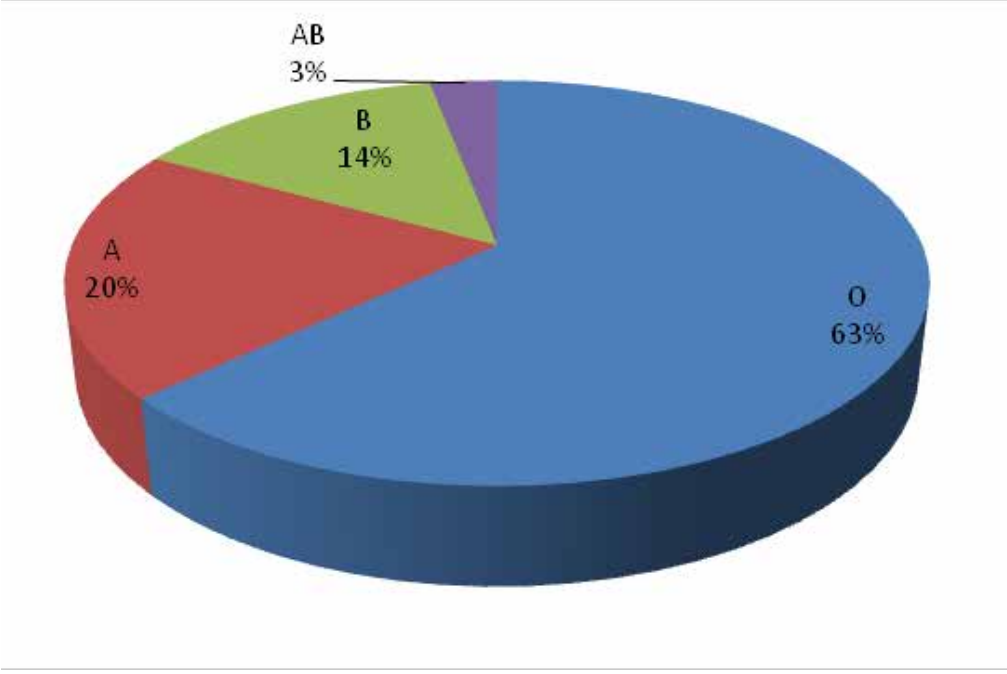

Figure 1: Prevalence of ABO blood group among pregnant women in UCTH

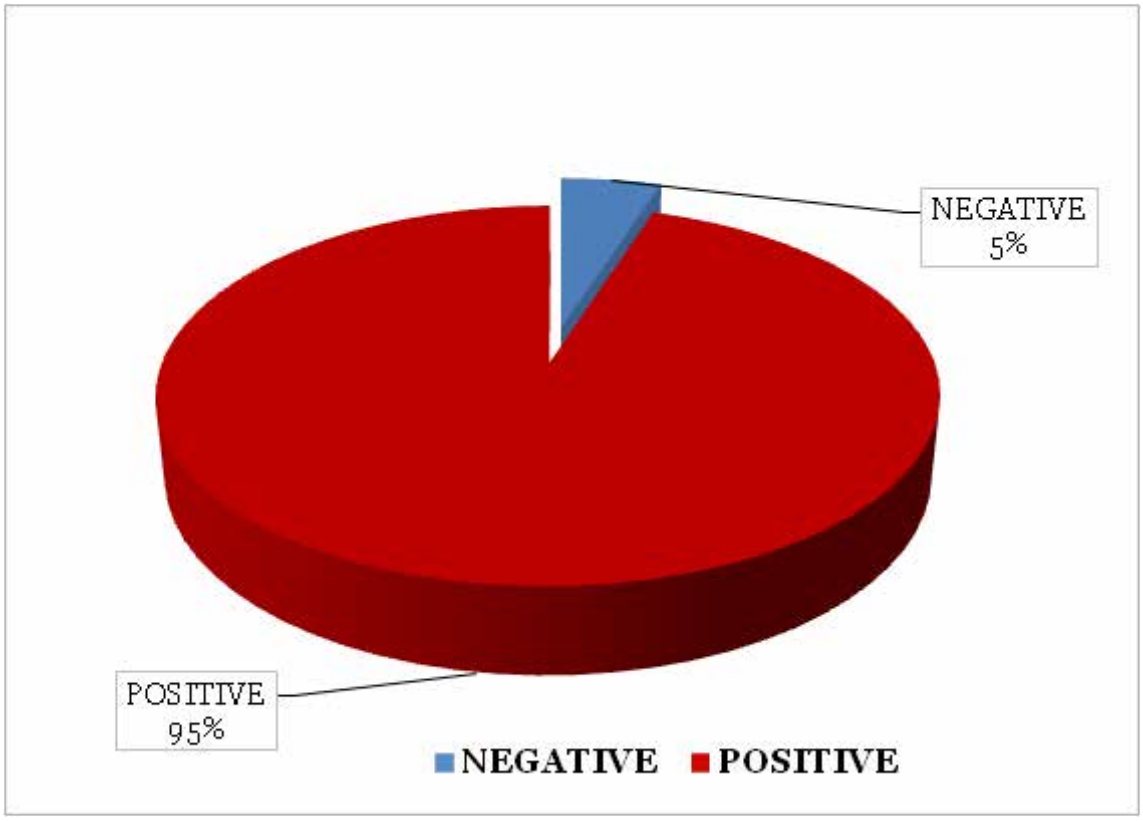

Figure 2: Prevalence of rhesus positive and rhesus negative antigens of pregnant women in UCTH

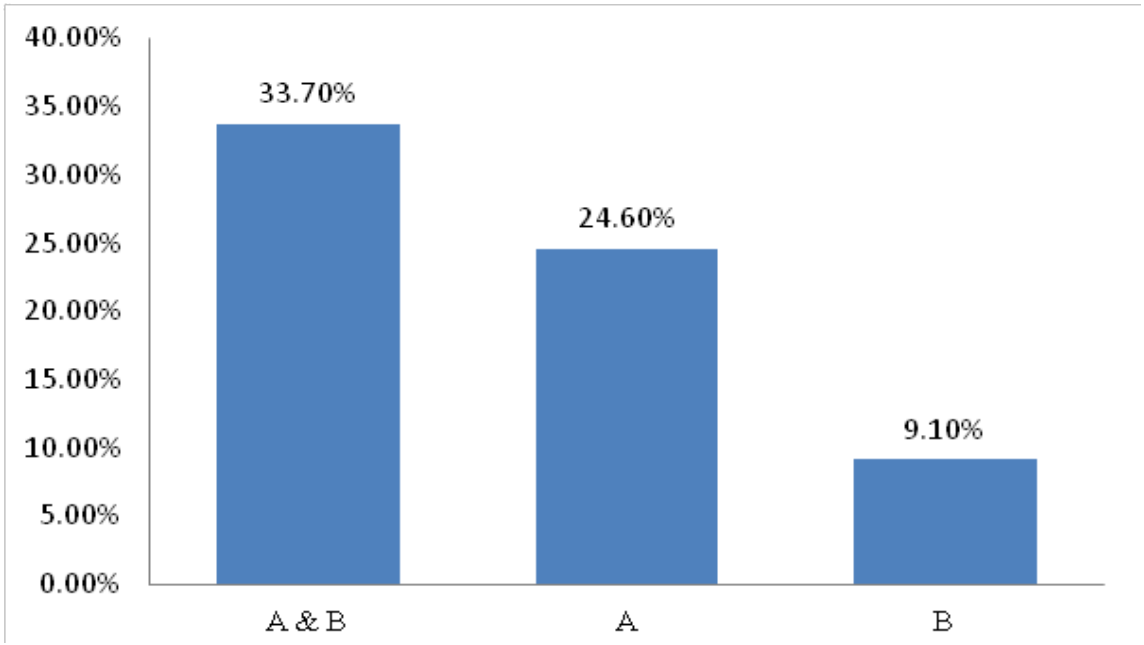

Figure 3: Percentage distribution of haemolysin in blood group O pregnant women

Table 2: Association between women who had stillbirth, miscarriage, transfusion and their ABO Blood Group

	ABO BLOOD GROUP				TOTAL n(%)	Statistics
	A n(%)	AB n(%)	B n(%)	O n(%)		
Stillbirth	76(20.1)	12(3.2)	52(13.8)	238(62.9)	378(100.0)	$\chi2$ =1.709 P = 0.635
Yes	4(18.2)	0(0.0)	4(18.2)	14(63.6)	22(100.0)	
Miscarriage						
No	72(21.7)	10(3.0)	46(13.9)	204(61.4)	332(100.0)	$\chi2$ =3.557 P = 0.313
Yes	8(11.8)	2(2.9)	10(14.7)	48(70.6)	68(100.0)	
Transfusion						
No	76(20.0)	12(3.2)	52(13.6)	240(63.2)	380(100.0)	$\chi2$ =1.203 P = 0.752
Yes	4(20.0)	0(0.0)	4(20.0)	12(60.0)	20(100.0)	

Table 3: Association between women who had stillbirth, miscarriage, transfusion and their Rhesus Blood Group

	Rh blood group		Total n(%)	Statistics
Stillbirth	Positive n(%)	Negative n(%)		
Yes	18(81.8)	4(18.2)	22(100.0)	$\chi2$ = 8.52
No	362(95.8)	16(4.2)	378(100.0)	P = 0.004
Miscarriage				
Yes	64(94.1)	4(5.9)	68(100.0)	$\chi2$ = 0.13
No	316(95.2)	16(4.8)	332(100.0)	P = 0.714
Transfusion				
Yes	18(90.0)	2(10.0)	20(100.0)	$\chi2$ = 1.11
No	362(95.3)	18(4.7)	380(100.0)	P = 0.293

Discussion

A woman's peak reproductive years are between the late teens and late 20. By age 30yrs the ability to get pregnant starts to decline, this decline becomes more rapid from 36yrs, and by age 45yrs fertility has declined so much that getting pregnant naturally is unlikely for most women as was observed in this study. There was a marked declined in the frequency of the pregnant women at age 45yrs and above which recorded 0.5%. This study agrees with that study reported by (11) that reported women of ages 16-35years belong to a large and diverse group of individuals who are at different stages in their reproductive life. The age and parity distribution of the cases in this study were also similar to those in other report, (12). Based on parity the study population was distributed as primigravidae, multigravidae and grandmultigravidae with a frequency of 62.5%, 35% and 2.5% respectively. The primegravidae constituted majority of the studied pregnant women.

Foeto-Maternal blood grouping incompatibility is known to contribute to still birth and miscarriage. Typical example of blood group system implicated in this manner are ABO, Rhesus, Kell, Duffy, Kidd, Lewis, MNS and P blood group antigens (13). Unfortunately the underlying causes of stillbirth and miscarriage often go undetected in resource poor settings such as ours. This study observed retrospectively the number of stillbirth and miscarriages. Approximately 5.5% of stillbirth and 17% miscarriages were recorded within the study period. However, majority of them had not received blood transfusion only 5% (14). Still birth is technically defined as foetal death at or after 20 to 28 weeks of pregnancy resulting in the baby being born without sign of life (15). Blood group O was predominantly seen among pregnant women who do experience or do not experience stillbirth, miscarriages and transfusion status during the course of the study.

Miscarriage prevalence of 17% was observed in this study, clinical miscarriage occurs when pregnancy loss occurs within six (6) weeks of gestation with histological and ultrasound evidence that poor intrauterine pregnancy has existed. Causes of miscarriage include immunological and immunogenetic (antigen-antibody, incompatibility) causes, endocrinological causes, sperm DNA fragmentation, uterine malfunctions, gene polymorphism causes and many others (16).

In this study blood group 'O' was the most predominant group occurring in 63% of the pregnant women, occuring about 3 times the frequency of each groups A (20%) and B (14%). Only 3% of the pregnant women, were of blood group 'AB'. The phenotype frequencies with respect to ABO in this study can be represented as O > A > B > AB. Although the distribution of ABO blood groups varies from one population to the other, in most studies blood group 'O' has been reported as the predominate group and blood group 'AB' as the least which agrees with this study and previous studies in Nigeria (17)(18). However slight disparity in distribution has been reported in study in Adamawa where blood group 'B' was more prevalent than Blood Group 'A' (19). The ABO prevalence in Nigeria varies slightly in different regions, but blood group 'O' has been reported as the most prevalent in all reported areas of studies.

In this study, blood group 'O' was found to be the most predorminate blood group among the pregnant women. This observation is a common finding in sub-Sahara Africa and is well documented (20). This observation is partially an advantage with respect to availability of blood for donation and transfusion. Blood transfusion in our environment usually follows the rule of transfusing only the same blood group to a recipient after a major cross-match. However, situations often arise when there is non-availability of specific donor blood, in emergency situation when there is no time to

wait for a cross match and neonate born to non ABO group identical mothers requiring transfusion of group O donor blood to recipients who are blood groups A, B, and AB (6) Group O is often more readily available in our blood banks. Transfusion of blood group O to non group O recipients is performed with the impression that blood group O donors are universal donors of red cells based on the fact that blood group O cells possess no AB-antigens, which the non group O recipient has antibodies against without taking in to consideration the presence of haemolysin in such donor blood. However, care "must" be taken while handling blood group O with the tag "Universal Donor" as some researchers has noted (7)(21). Some blood group 'O' do contain alpha (α) and beta (β) haemolytic antibodies (haemolysin). which might poses a risk of alloimmuniation in non-blood group O individual especially a blood group "O" mother with a blood group A foetus, the Anti A from the mother attacks the Foetal A cells with subsequent lysis, with likely incidence of ABO incompatibility resulting in (HDN) Haemolytic diseases of the new born (22). In this study, approximately 252 blood Group O haemolysin was carried out, haemolysin α+β, recorded (33.7%), haemolysin α-(24.6%) and Haemolysin β – (9.1%). This is also comparable with study reported by (23) in Ilorin 2011 with a prevalence of 32.2%, 38.1% and 23.2% respectively.

Erhabor et al. (21), noted that Plasma negative for haemolysin could be given to those with group A, B and AB while that with positive haemolysin be reserved for group O individuals. As this may be the result of the immune antibodies of the ABO system observed in this studies. However, Anyanwu and colleagues reported prevalence of 16.7%, 11.1% and 16.7% of α, β, and α +β haemolysin respectively, for sickle cell patients in Calabar (20). This difference in prevalence obtained by various studies in Nigeria may be due to methodology. The serum- cell ratio has been documented to affect outcome of haemolysis analysis; higher serum-cell ratio has tendency for red cell lysis. This high frequencies of alpha and beta haemolysin has been reported to be responsible for the high frequencies of ABO – haemolytic disease of the new born seen in African (23).

Rhesus blood group system is highly polymorphic and is the second most important in transfusion medicine after the ABO. prevalence of Rh(D) positive in this study was 95% while that of Rh negative was 5%. This sequence is similar to 91.6%, 88.2% and 97.1% being studies done in Calabar, Adamawa and Kano respectively (24)(25)(19). Rhesus positive blood group pregnant women attending antenatal showed to be more predominant when occurrences of stillbirth, miscarriages and transfusion status was considered. In this study antenatal screening for various blood group systems was generally targeted solely at detection of anti-D in Rh negative women and routine antibody screening was done for Rh-D negative women alone. Some of the reports from other studies like in India have described allo antibodies in Rh-D positive women (26). In our study 0.5% allo antibodies were observed which included both Rh positive (95%) and Rh negative 5% therefore antibody screening of both Rh positive and negative women is required.

Conclusion

The data collected revealed that ABO blood group distribution was in the order O>A>B>AB 63%, 20%, 14% and 3.0%. Blood group O was seen to occur three times more than the other blood groups. On the other hand, the study shows the prevalence of Rhesus D' positive and Rhesus D' negative to be 95% and 5% respectively. Blood group 'O' and rhesus positive blood group were predominant in stillbirth, miscarriages and transfusion status.

There is a need to routinely screen for haemolysin so as to prevent a potential risk, of immune reaction. It is recommended that the use of group O blood free from haemolysin α and β should only be transfused to pregnant women because of risk to the foetus.

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