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Seroprevalence of Rhesus antigens, genotype distribution and its relations with ABO groups among Pregnant Women in University of Calabar, Calabar, Nigeria

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Abstract

The Rhesus blood group system consists of six genes: *Cc*, *Dd*, *Ee*. At least 36 Rhesus genotypes are possible based on the combinations of genes that can be inherited (Agarwal *et al.,* 2014). This study is aimed at providing information on the seroprevalence of Rhesus genotype distribution and its association with ABO group among pregnant women in University of Calabar Teaching Hospital, Calabar Nigeria. A descriptive cross-sectional study comprising of 400 pregnant women who gave their informed consent were recruited. Whole blood and serum were collected and used respectively for the study. Rhesus blood group was analysed using a commercially prepared reagent. Antibody screening was performed using standard cells. Antibody identification was done using panel cells. Frequency, percentage and chi-squared statistical tools were used. Most of the pregnant women were aged 16 – 36 years and primegravidae constituted majority of subjects recorded in this study. The prevalence of Rhesus positive and Negative was 95% and 5% respectively. Seropositive was anti-C 88 (22.0%), anti-c 394 (98.5%), anti-E 106(26.5%), and anti-e 394 (98.5%), while seronegative was anti-C 312(78.0%), anti-c 6(1.5%), anti-E 294(73.5%), anti-e 6(1.5%) respectively. Percentage distribution of Rhesus phenotype among the pregnant women was found to be R^0R^0 (51.50%) followed by R^2R^0 (22%) and $R'R^0$ (17.5%), while the least were r'r, R^2R^2, R^2R', ryry and R'R', which recorded 0.50%. The most prevalent possible genotype was cDe/cD while CdE/CdE had the least genotype found in blood group A. The negative Rhesus genotype cde/cde (rr) was found in 2.5% of the studied population and found in blood group O alone. Rhesus phenotyping should be included as routine screening test and all pregnant women should be screened irrespective of their Rhesus status.

Keywords: *Rhesus phenotypes, pregnant women, Calabar*

Introduction

Pregnancy, also known as gravidity or gestation, is the time during which one or more babies develop inside a woman (Obrowski *et al.,* 2016). After the ABO blood group system, the second most significant blood group system is Rhesus system. The system was first discovered when a mother of a still born foetus suffered a severe haemolytic reaction when transfused with her husband's blood. The mother who obviously lacked some new antigen must have been immunized by her foetus that possessed this antigen (Sankaralingam *et al.,* 2016).

The Rhesus blood group system consists of six genes: *Cc*, *Dd*, *Ee*. A single chromosome can carry *C* but not *c*, *D* but not *d*, and *E* but not *e*. A person inherits from each parent a set of three closely positioned Rhesus genes, e.g. *CDe*/*cde*. At least 36 Rhesus genotypes are possible based on the combinations of genes that can be inherited (Agarwal *et al.,* 2014). When using the Fisher-Race derived nomenclature, Rhesus antigens bear the same name as their genes, i.e. antigens D, C, c, E and e (*d* gene is not expressed). Rhesus antigens are only expressed on red cells. They are not found in body fluids (Flegel, 2007).

The Rhesus factor is clinically the most important protein-based blood group system. With 49 antigens so far described, it is the largest of all 29 blood group systems. The unusually large number of Rhesus antigens is attributable to its complex genetic basis. The antigens are located on two Rhesus proteins - RhD and RhCE - and are produced by differences in their protein sequences. In CD nomenclature, they are termed CD240D and CD240CE unlike proteins of other blood groups (Kumar *et al.,* 2014). The Rhesus factor is clinically the most important protein-based blood group system. With 49 antigens so far described, it is the largest of all 29 blood group systems. The unusually large number of Rhesus antigens is attributable to its complex genetic basis. The antigens are located on two Rhesus proteins - RhD and RhCE - and are produced by differences in their protein sequences. In CD nomenclature, they are termed CD240D and CD240CE unlike proteins of other blood groups (Kumar *et al.,* 2014).

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 in resolving cases of doubtful parentage. Cross River State, Nigeria is a vast territory with many ethnic groups (Onor, 2016); 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

Subjects for this study consisted of four hundred (400) pregnant women attending the antenatal clinic at University of Calabar Teaching Hospital. This study was carried out over a period of six (6) months, and the subjects (pregnant women) were aged 16-45years in their first, second or third trimester. The following socio-demographic characteristics including parity, history of gravidae outcome and transfusion history were extracted from the patients record. Ethical clearance was obtained from Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital, with reference number UCTH/HREC/33/660.

Inclusion and exclusion 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 while 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 for the prospective study were collected from the pregnant women who came for antenatal clinic at UCTH.

A total of three (3) millitres 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 antibody screening and antibody identification type. The red cells were grouped/ typed for Rhesus and Rhesus phenotype identifications.

Determination of Rhesus blood group

A. Rhesus grouping

Rhesus grouping was done using Anti-D monoclonal reagent bought from Biotec (Dacies and Lewis, 2008).

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 2 hours.
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 seconds at 1000rpm the cell was gently re-suspended and observed macroscopically and microscopically for agglutination.
   Rhesus Negative and positive controls were included in all procedures.

Materials & method for antibody screening and identification

Screening and identification of red cell all antibodies were done on the serum of 400 pregnancy women using Diacell, Diapanel reagent (Lorne Laboratories Ltd, Great Britain).

B. Antibody screening and identification Principle of the procedure; involve testing unknown serum against a set of group O reagent red cells that together contain most of the antigens necessary to detect clinically significant antibodies.

i. **Antibody Screening Panel 3 Cells**
   5 Cells pooled from group O individual.
   5 Cell pooled from group O Negative (Negative control).
   5 cells pooled from group O Negative Sensitized (Positive control).

i. 1 volume of sera was dispensed into clean test tube respectively.
ii. 1 volume of screening panel cells was added into the tube.
iii. Mixed and incubated at 37^{oc} for 1½hours.
iv. 1 volume of 3% Albumin was added to it and incubate for 2 hours
v. Cell was washed and AHG 1 volume added.
vi. Spin for 20seconds at 1,000rpm
vii. The suspension was examined macroscopically and microscopically for agglutination.

Result: Agglutination present: Antibody Screening Positive.

No Agglutination present: Antibody Screening Negative.

C. Antibody Identification

Identification panel (8 cells identification). From Diamed (UK) was used to identify allo-antibodies type by tube method in low ionic strength solution, Albumin and AHG.

1 volume of the positive screening sera was dispensed into a clean test tube of 8 tubes.

i. The identification panel cells 1 volume each was added to the positive sera respectively.
ii. The suspension was mixed and incubated at 37^{oc} for one hour
iii. 1 volume of Albumin was added, washed and 1 volume AHG was added, and it was spin slightly for 20 seconds and agglutination was observed.
iv. For specific antibody type using the identigram provided.
v. The reaction patterns were evaluated to identify the antibodies present.
vi. To ensure quality control the manufacturer's instructions was strictly adhered to.
vii. Each batch of test was run with both negative and positive control.
viii. Auto control was also set up in other to rule out the presence of auto antibody.

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. Most of the study subjects were in the aged group 16 – 25years 178 (44.5%), 26 – 35years 174(43.5%), 36 -45years 48(12.0%), >46 2(0.5%). It also showed the study subjects based on parity, the study population were distributed as primigravidae (n=250), Multigravidae (n=140) and grand Multigravida (n=10). The percentages were observed to be 62.5%, 35% and 2.5% respectively. Transfusion status (n=20) and number of times of transfusion percentages were 5% and 5% respectively.

Figure 1 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.

Table 2 shows the prevalence of the various blood group antigens studied among the pregnant women in UCTH. In the 400 pregnant women recruited, the prevalence of anti-C positive was found to be 88 (22.0%), anti-C negative was 312 (78.0%), anti-c positive 394 (98.5%), anti-c negative 6 (1.5%), anti-E positive 106 (26.5%), anti-E negative 294 (73.5%), anti-e positive 394 (98.5%), anti-e negative 6 (1.5%).

Figure 2 displays the percentage distribution of Rhesus genotype among pregnant women in UCTH. cDe/cDe had the highest prevalence of 51.5% (206/400) while cdE/cde, cDE/cDE, cDE/CDe, CDe/CDe and CdE/CdE had the least percentage distribution of 0.5% (2/400) respectively.

Table 3 shows the Rhesus genotype among pregnant women of different ABO blood group. Blood group A were 80 in number of which 38 (47.5%) belonged to cDe/cDe, followed by CDe/cDe 20 (25%), 18 (22.5%) had cDE/cDe, then cDe/CDe and CDe/CDe 2 (2.5%). None of blood group A pregnant women had the following rhesus genotypes cde/cde, cdE/cde, cDE/cDE, cDE/CDe, Cde/cde, CDe/CDe and CDE/cDe. A total of 56 pregnant women belonged to blood group B having cDe/cDe, had a frequency of 36 (64.3%) followed by cDE/cDe 10 (17.9%) CDe/cDe 6 (10.7%) Cde/cde 4 (7.1%) while the following Rhesus genotype cde/cde, cDe/CDe, cDE/cDE, cDE/CDe, CdE/CdE, CDe/CDe and CDE/cDe) were not found in blood group B. Blood group AB pregnant women were 12 in number with 83.3% belonging to cDe/cDe while 2 (16.7%) belong to Cde/cDe. Other Rhesus genotypes were not found in blood group AB. Blood group O subjects were 252 and fully represented in the various Rhesus genotypes. cDe/cDe had a frequency of 122 (48.4%) cDE/cDe 60 (23.8%) CDe/cDe 42 (16.7%) cde/cde, Cde/cde and CDE/cDe had the same frequency of 6 (2.4%) and the least cdE/cde, cDe/cDe, cDE/cDE, cDE/CDe, and CDe/CDe 2 (0.8%) each respectively. Only rhesus genotype CdE/CdE was not found in blood group O and was the only genotype found in blood group A.

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		
Primigravidae	250	62.5%
Multigravidae	140	35%
Grand multigravidae	10	2.5%
TRANSFUSION STATUS		
YES	20	5.00
NO	380	95.00
NUMBER OF TIMES TRANSFUSED		
1	20	5.00
NONE	380	95.00

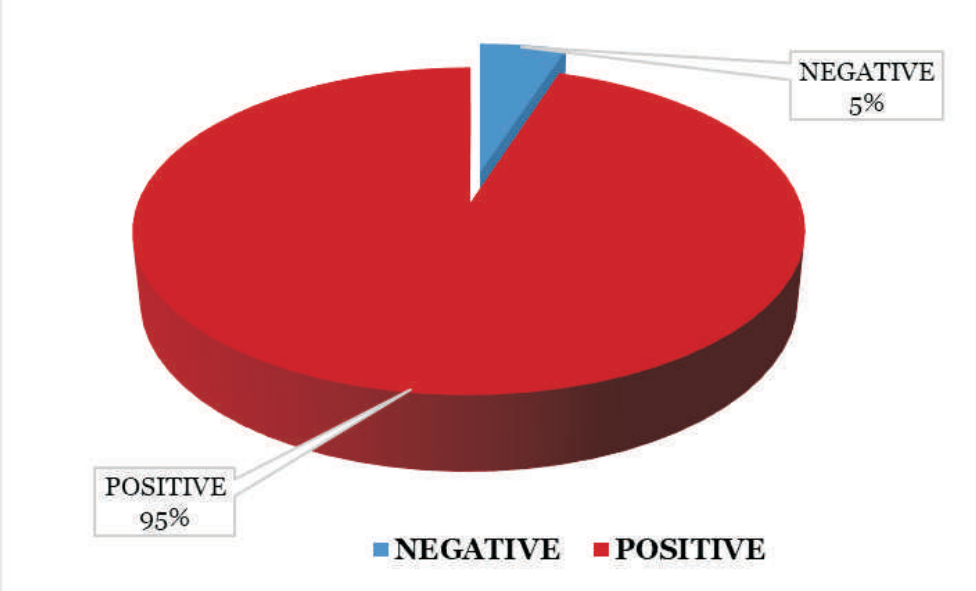

Figure 1: Prevalence of Rhesus positive and rhesus negative antigens of pregnant women in UCTH

Table 2: Prevalence of antigens of the Rhesus blood group systems among pregnant women in UCTH

Result	RhD	C	C	E	E
Negative (%)	20 (5.0)	312 (78.0)	6 (1.5)	294 (73.5)	6 (1.5)
Positive (%)	380 (95.0)	88 (22.0)	394 (98.5)	106 (26.5)	394 (98.5)

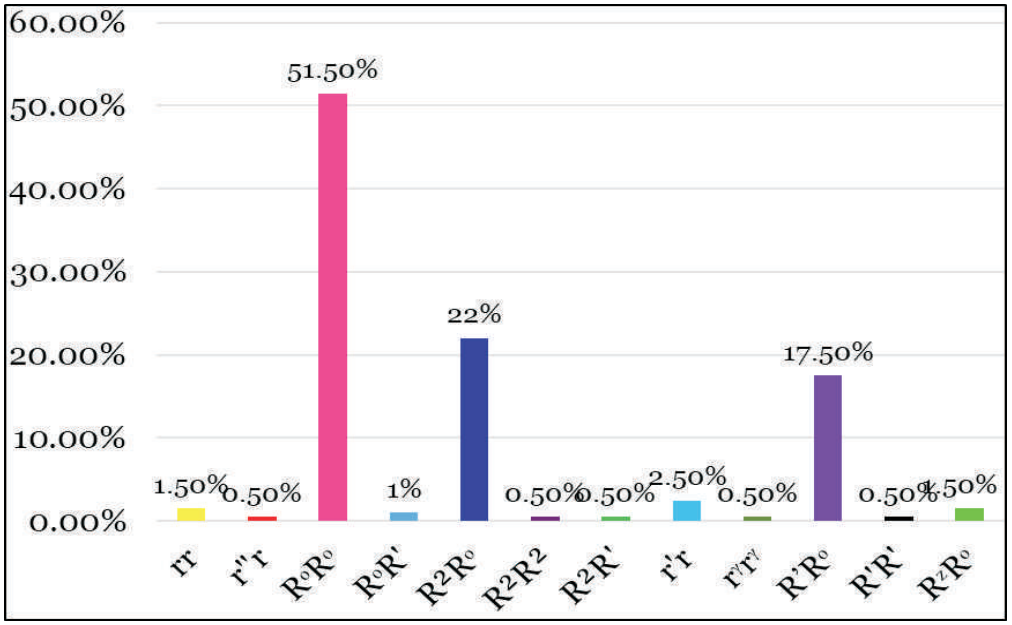

Figure 2: Rhesus genotypes of pregnant women in UCTH

Table 3: Showing the Association between Rh genotypes and ABO Blood Group

Genotype	A	AB	B	0	Total	Statistics
cde/cde	0	0	0	6	6	$X^2 = 41.941$
cdE/cde	0	0	0	2	2	
cDe/cDe	38	10	36	122	206	df = 33
cDe/CDe	2	0	0	2	4	
cDE/cDe	18	0	10	60	88	P = 0.029
cDE/cDE	0	0	0	2	2	
cDE/CDe	0	0	0	2	2	
Cde/cde	0	0	4	6	10	
CdE/CdE	2	0	0	0	2	
CDe/cDe	20	2	6	42	70	
CDe/Cde	0	0	0	2	2	
CDE/cDe	0	0	0	6	6	
Total	80	12	56	252	400	

There is a significant statistical association between Rhesus Phenotype and ABO blood group X^2 (33) = 40.941, p = 0.029.

Discussion

The age and parity distribution of the cases in this study were also similar to those in other reports (Bae *et al.,* 2011; Oh and Bae, 2019). The primigravidae constituted majority of the studied pregnant women. The 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 consistent with a prevalence of 91.6%, 88.2% and 97.1% observed in previous studies done in Calabar, Adamawa and Kano (Gwaram and Abdullahi, 2013, Kooffreh *et al.,* 2015; Etim *et al.,* 2017). The frequency of other Rhesus antigens in our study subjects were as follows: c, 98.5%; C, 22%, e, 98.5%; and E, 26.5%. Although anti D is the most potent and immunogenic, the other antigens in the system can sensitize the immune system to give rise to clinically significant antibodies when Rhesus incompatible (D, c, C, e, & E antigen D) units are transfused to patients. Comparatively the most prevalent Rhesus antigens were c and e followed by D. This observation is similar to the report from a study in Port Harcourt where the antigen c had the highest prevalence of 100% (Jeremiah *et al.,* 2011).

Antibodies specific to this system are the most frequent antibodies encountered in pre-transfusion testing and is the main cause of haemolytic disease of newborn. This study has shown that other Rhesus antigens are prevalent. Rh c and e (98.5%) are more prevalent than Rhesus D. among the pregnant women in UCTH and the least was C 22%. The distribution of the Rhesus antigens follows the order; c, e, > D > E > C. This report agrees with Suleiman *et al*. (2011) which stated that the distribution of Rhesus blood group antigens in Zaria showed the

commonest antigen as the c antigen with a prevalence of 98.44% while the least common antigen was the C antigen, with 10.16% which is at variance with my findings with C, 22%. Our finding is also consistent with previous report by Erhabor *et al.* (2016) among pregnant women in Sokoto Nigeria which indicated a prevalence of Rh c was 92% while Rh e was 98.5%. Also, our observed prevalence of C antigen of 22% is consistent with a prevalence of 25.8% observed among pregnant women in Sokoto Northwestern Nigeria by Erhabor *et al*. (2015). However, the result is dissimilar to studies in Kano and Maiduguri where antigen D was reported as the most prevalent (Kagu *et al.,* 2011; Gwaram *et al.,* 2013). This may be due to racial differences and genetic distribution which could be because of immigration from the border. The implication of this finding of high prevalence of antigens c, e and D is the probable result of low incidence of immunological reactions that occur due to lack of antigens especially D. Rhesus D is among the chief causes of haemolytic disease of the newborn. On the dark side this translates to scarcity of Rhesus D negative blood when the need arises (Gwaram *et al.,* 2013).

It was also observed from this study that to a higher extent Rhesus antigen can be used to determine the Rhesus phenotypes and its likely genotypes. This observation agrees with the report of Jeremiah *et al.* (2011). This is also in agreement with Sharma *et al*. (2013) who stated that a probable genotype may be speculated on, based on the statistical distributions of Rhesus Phenotypes in the patient's place of origin. It also agrees with Dacie and Lewis, which stated that determination of Rhesus Phenotype of an individual and its likely genotype has a common reason which is to detect whether an individual is homozygous for a particular Rhesus antigen (Dacie and Lewis, 2008).

In this study the distribution of possible Rhesus genotype of the pregnant women studied showed cDe/cDe as the highest frequency of 51.5%, cde/cde had 5% while cdE/cde, had the least frequency of 0.5%. A true Rh-negative individual will have the phenotype cde/cde. From this study it was observed that 20(5%) pregnant women were Rh negative but from the possible genotype only 10 (2.25%) were true Rh negative and was found in blood group O pregnant women only. Out of the eighteen (18) positive (4.5%) for antibody screening only six (6) antibodies were detected, antibodies other than Anti-D were identified: Anti D+C (n=1; 0.25%), Anti D (n=3; 0.8%), Anti-C (n=2; 0.5%). Similar incidence has been observed in reports from Nigeria (Jeremiah *et al.,* 2011). This may be attributed to better access of healthcare services and the total number of women screened and the duration as compared to other studies with a longer duration of screening process thereby increasing their sample size.

Conclusion

The Rhesus antigens were seen in this order c>e>D>E>C. The most prevalent possible genotype was cDe/cD while CdE/CdE had the least genotype found in blood group A. The negative Rhesus genotype cde/cde was found in 2.5% of the studied population and found in blood group O alone.

1. Rhesus phenotyping should be included as routine screening test in the blood transfusion centre in UCTH, and all pregnant women should be screened irrespective of their Rhesus status.

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