https://doi.org/10.46344/JBINO.2023.v12i02.06

PREVALENCE OF RHESUS C AND Du PHENOTYPES AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT BOWEN UNIVERSITY TEACHING HOSPITAL (BUTH) OGBOMOSO, NIGERIA

Arinze Favour Anyiam [1], Ejeatuluchukwu Obi[2], Onyinye Cecilia Arinze-Anyiam[1], Emmanuel Anyachukwu Irondi [3], Pius Omoruyi Omosigho[4], Musa Abidemi Muhibi[4], Ayodele Ilesanmi [5], Akintola Ayodeji Stella [5], Blessing Okezie [6] and *Emmanuel Ifeanyi Obeagu[7]

[1]*Department of Medical Laboratory Science, Haematology Unit, Faculty of Basic Medical and Health Sciences, Thomas Adewumi University, Oko, P. M. B. 1030, Kwara State, Nigeria.*
[2] *Toxicology Unit, Department of Pharmacology and Therapeutics, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria*
[3] *Department of Medical Biochemistry and Pharmacology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, P.M. B. 1530, Kwara State, Nigeria.*
[4]*Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Edo State University, Uzairue, Edo State, Nigeria.*
[5] *Department of Medical Laboratory Science,, Faculty of Pure and Applied Sciences, Kwara State University, Malete, P.M. B. 1530, Kwara State, Nigeria.*
[6] *Department of Medical Laboratory Science, Achiever's University, Owo, Ondo State, Nigeria.*
[7]*Department of Medical Laboratory Science, Kampala International University, Uganda.*

ABSTRACT

The aim is this study is to determine the prevalence of Rh c and Du phenotype among pregnant women attending antenatal clinic (ANC) in Bowen University Teaching Hospital Ogbomoso. The prevalence and distribution of Rh C and Du phenotype was determined using 50 blood samples of 50 different pregnant women consecutively recruited pregnant women aged 18-40 years. Samples were tested for Rh C and Du phenotype using the conventional tube agglutination method using Rapid Laboratories (UK) anti C and Du antisera. Out of 50 samples studied, the prevalence of Rh C was 28% (positivity) while Rh Du was 92% (positivity). The prevalence of Rhesus Du antigens was higher in Ogbomoso since 46 out of 50 samples (92%) tested positive for Rhesus Du antigens. In addition, a 28% prevalence (positivity) obtained does not postulate that Rhesus C antigen distribution in pregnant women in Ogbomoso is low. We recommend that all pregnant women in the area be screened for the presence of clinically significant red cell antigens including Rh C and Du blood group antigens on their first antenatal visit.

Keywords: *prevalence, rhesus C, Du phenotypes, pregnant women*

INTRODUCTION

Rhesus (Rh) antigen was discovered in 1940 by Karl Landsteiner and Wiener. In later years, because of its immunogenicity along with ABO antigens, the Rh blood group system is second most clinically significant blood group system after the ABO blood group system. Rhesus antigen or Rh factor is a certain type of protein found on the surface of red blood cell. The protein is genetically inherited. 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 [1-10].

Rh phenotypes are readily identified through the presence or absence of the Rh surface antigens. Blood group antigens play a vital role in transfusion medicine, genetics understanding, inheritance pattern, and disease susceptibility [11]. The benefit of knowledge of the blood group pattern in transfusion services is the reducing of maternal mortality rate and useful in clinical practice, because in certain conditions an antigen may react with its corresponding antibody and cause serious clinical effects like hemolytic disease of the newborn and hemolytic transfusion reaction. Therefore, it is fundamental to have information on the distribution of these blood groups in any population group. The Rh antigens are highly immunogenic, and most of the rhesus antibodies should be considered potentially capable of causing hemolytic transfusion reactions and hemolytic disease of the fetus and newborn [12-13]. Anti-D causes the most severe form of HDN and it used to be a major cause of fetal death. However, all cases cannot be prevented, and RhD alloimmunization remains a major cause of disease [14]. Anti-c Rh alloantibodies are also capable of causing severe HDN [15] which is considered the most important Rh antigen after the D antigen. Rhesus allo-antibodies that areassociated with mild HDN include anti-C anti-E and anti-e [16-17].Anti-D, anti-C, anti-E, and anti-e have all been involved in delayed hemolytic transfusion reactions. The prevention of Rhesus D Factor (RhD) alloimmunization in higher income countries remains one of the most important medical accomplishments of the last century [18-20]. The prevention program involves the administration of Rh immune globulin (RhIG), both antenatally and post-natally, and remains the gold standard in effective prevention [21-24]. Prior to the development of a prophylaxis, families suffered the loss of the fetus as a consequence of Hemolytic Disease of the Fetus/Newborn (HDF/N). The discovery of the antibody formation in Rh D negative women toward RhD positive fetuses was a breakthrough in the identification of DF/N [17, 25]. Once the mechanism facilitating HDF/N onset was determined, the development of a prophylaxis, Rh immunoglobulin, followed soon after [17].

The aim of the study was to ascertain the prevalence of Rhesus C and Du phenotypes among pregnant women attending antenatal clinic in Bowen University Teaching Hospital Ogbomoso, Nigeria.

MATERIALS AND METHODS

Study area

The research work was carried out in Bowen University Teaching Hospital, Ogbomoso, Oyo State.

Selection of Subjects

Subjects for this study comprised of consenting pregnant women (between 18 and 40 years) attending antenatal clinic at Bowen University Teaching Hospital Ogbomoso, (BUTH), Ogbomoso.

Inclusion criteria

Pregnant women on neonatal visit, with age range of 18-40 years and those with

blood transfusion history were selected for the research.

Exclusion criteria

Pregnant women with the age group less than 18 years and more than 40 years were excluded from the research work.Also, pregnant women who did not give the consent were excluded from the study.

Ethical consideration

Approval for this study was obtained from the Ethical Review Committee of BUTH. The sample collection was explained to the patient using the information sheet provided in written informed consent. Each patient was required to give a written informed consent before being eligible to participate in the study.

Sample Size Determination

The sample size will be obtained using the formular of Naing *et al.*(2008) and stated thus:

Rhesus Du Antigen :

$N = Z^2 X P (1 – P)/ d^2$

N = Minimum sample size

P = Prevalence of Du (3.4%) in pregnant women in Nigeria (Gwaram and Abdullahi, 2013).

d = Desired level of significance = 0.05 (5%)

Z = Confidence interval = 1.96 (95% confidence interval)

$N = (1.96)^2 X\ 0.034(1 – 0.034)/ (0.05)^2$

N = 3.8416 X 0.032844/ 0.0025

N = 45 approximately 50 (1 significant figure)

Rhesus C Antigen:

$N = Z^2 X P (1 – P)/ d^2$

N = Minimum sample size

P = Prevalence of Rhesus C (28%) in pregnant women in Nigeria (Gwaram and Abdullahi, 2013).

d = Desired level of significance = 0.05 (5%)

Z = Confidence interval = 1.96 (95% confidence interval)

$N = (1.96)^2 X\ 0.28(1 – 0.28)/ (0.05)^2$

N = 3.8416 X 0.032844/ 0.0025

N = 309 approximately 300 (1 significant figure)

Data Collection

Data from collected blood samples was collated and analyzed statistically.

Specimen Collection

After aseptic washing with 70% ethyl alcohol, blood samples were collected into an EDTA bottle. Five milliliters of whole blood were collected using a syringe and needle into EDTA anti coagulated tube. EDTA anti-coagulated blood was kept at +4°C36

Laboratory Investigation

Rhesus C Determination

The collected blood sample is used for the determination of Rhesus phenotype using Rapid Laboratories anti-C reagents. A drop of blood from each volunteer was placed on a glass slide in three places. A drop of each of the anti-C antisera was added and mixed with each blood sample, with the aid of glass rods.

Rhesus Du Determination

A washed 3% suspension of patient cells was prepared, later the Du antigen and anti-C (Rh Control) tubes were set up, if not already done. The Du and anti-C immediate spin results were recorded. When the Rh test was negative, the initial step was continued. Incubated both tubes at 37°C for 15 to 30 minutes. Following centrifugation, agglutination was read for as usual. When the Rh test is negative, it was continued by washing both tubes 3-4 times with saline. Immediately after the last wash, one-drop Coombs serum was added to each tube and centrifugation follows in the serofuge the time appropriate for the Coombs spin calibration. Immediately resuspended gently and examined for agglutination using the lighted agglutination viewer. Results were recorded in the appropriate column on the worksheet Confirm all negative results by adding one drop Coombs control cells to all tubes showing no agglutination and centrifuge 15-30 seconds at high speed in the serofuge.

Suspension and examination for agglutination were later done. Agglutination should be present in this step or the test is invalid [26].

RESULTS

A total of 50 blood samples were collected from pregnant women aged 18 to 40 years with mean age 27.19 ± 4.70. They were attending Antenatal clinic in BUTH Ogbomoso. The design is such that an age interval of 6 years distributed the subjects into 5 groups as shown below

Table 1: Age Distribution of the subjects

Age Group (Years)	Frequency in distribution	Distribution (%)
15-20	4	8
21-25	4	8
26-30	24	48
31-35	14	28
36-40	4	8

Age group (6 years interval) was designed as shown in the table above. Each age group is sought into its frequency and the percentage distribution for respective frequency is calculated. Age group (26-30 years) has the highest percentage distribution (48%).

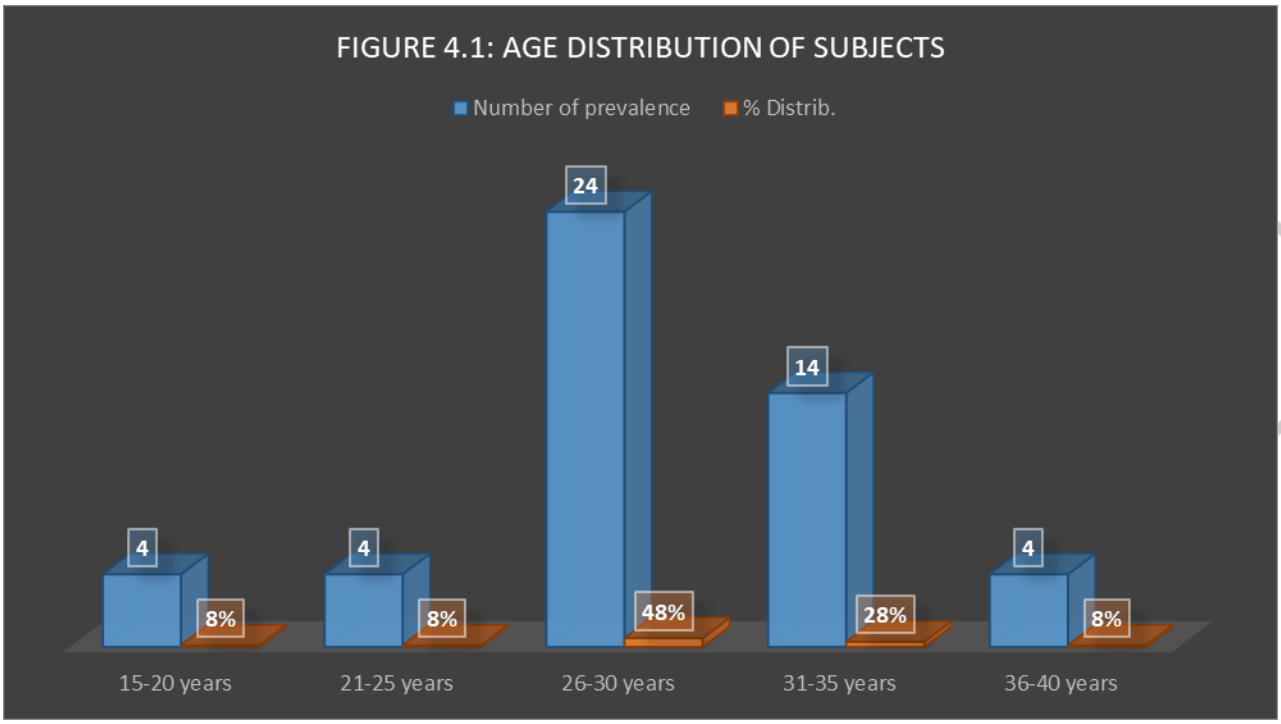

Figure 1: The Figure above shows the graphical presentation of the Age group distribution among the subjects. In age group (26-30 years), there were 24 subjects (equaling 48% of the 50 samples). Meanwhile in the age groups (15-20 years, 21-25 years and 36-40 years), there were 4 subjects each (each equaling 8% of the 50 samples).

The mean of the Rhesus Du antigen (positive prevalence) was significantly higher (9.20± 3.81) than the negative prevalence of Rhesus antigen (0.80± 0.37) ($p<0.05$). There was no statistical significance in the mean levels of the two Rhesus antigen status. In rhesus (C) antigen (positive prevalence), $p=0.0831$ ($p>0.05$), in

rhesus (C) antigen (negative prevalence), p=0.4327 (p>0.05).

Table 2 shows the prevalence of Rh Du antigens in the subjects

Rhesus (Du) Antigen status	Mean ± SEM	N	P
Rhesus (Du) antigen (positive prevalence)	9.20± 3.81	5	0.0831
Rhesus (Du) antigen (negative prevalence	0.80± 0.37	5	0.4327

Values are expressed as mean ± SEM. P-value is significant at 0.05, N= 5

Key:

SEM= Standard error of mean, p-value =0.05 (CI = confidence interval=95% or 0.95), N= number of groups=5, Test= 2 way ANOVA.

Table 3: Percentage prevalence of Rhesus Du among subjects

Rh (Du) Antigen Status	Number of prevalence	% Prevalence
Positive	**46**	**92**
Negative	**4**	**8**
Total	**50**	**100**

The percentage prevalence of Rh Du (Positive and Negative antigen status) were presented in the table. A total number of 46 samples of blood (92%) were counted for tubes (each containing a specific blood sample) showing agglutination with anti-Du reagent. These are taken as positive samples for Rhesus Du antigens. Percentage prevalence of 8% was recorded in the remaining 4 samples not showing agglutination with anti-Du reagents.

Table 4: shows the prevalence of Rh C antigens in the subjects.

The mean of the Rhesus Du antigen (positive prevalence) was significantly higher (2.80±0.97) than the negative prevalence of Rhesus antigen (7.20±3.06) ($p<0.05$) though there was no statistical significance in the mean levels of the two Rhesus antigen status. In rhesus (C) antigen (positive prevalence), p=0.1084 (p>0.05), in rhesus (C) antigen (negative prevalence),p=0.1258 (p>0.05).

Rhesus (C) Antigen status	Mean ± SEM	N	P
Rhesus (C) antigen (positive prevalence)	2.80±0.97	5	0.1084
Rhesus (C) antigen (negative prevalence	7.20±3.06	5	0.1258

Values are expressed as mean ± SEM. P-value is significant at 0.05, N= 5

Key:

SEM= Standard error of mean, p-value =0.05 (CI = confidence interval=95% or 0.95), N= number of groups=5, Test= 2 way ANOVA.

Table 5: Percentage prevalence of Rhesus C antigen among subjects

Rh (C) Antigen Status	Number of prevalence	% Prevalence
Positive	**14**	**28**
Negative	**36**	**72**
Total	**50**	**100**

The percentage prevalence of Rh C (Positive and Negative antigen status) were presented in the table. A total number of 14 samples of blood (28%) were counted for tubes (containing a specific blood sample) that agglutinated with anti-Du reagent. These are taken as positive samples for Rhesus C antigens. Percentage prevalence of 72% was recorded in the remaining 4 samples not showing agglutination with anti-Du reagents.

Discussion

Aside the ABO blood group system, the Rhesus blood group system is the second most clinically significant as reported by Avent [1]. The Rh system is postulated to be involved in hemolytic disease of the fetus and new-born, hemolytic transfusion reaction and in autoimmune hemolytic anemia and in forensic work. The determination of Rhesus C and Du status is of critical importance in the field of transfusion to prevent hemolytic transfusion reaction and obstetric medicine to prevent HDFN [27]). Currently,in Ogbomoso, pregnant women are only routinely tested for their ABO and Rh (D). Rhesus (C) and Du phenotype testing are not regularly done. Also the Rhesus phenotyping of blood donated for transfusion purposes in Ogbomoso are not routinely phenotyped for antigen C and other clinically significant red cell antigens. The Rhesus C phenotypes of pregnant women in the area are not well known. Pregnant women who are Rhesus C negative and are married to Rhesus C positive men run the risk of carrying a Rhesus C positive baby which can potentially put their mothers at risk of production of C antibody following any sensitizing events during pregnancy or delivery. This antibody-C can put future C positive pregnancies at risk of Hemolytic Disease of the Fetus and Newborn (HDFN).Similarly anemic pregnant women who are antigen C negative run the risk of being transfused with ABO compatible C positive donor units. This can potentially put them at risk of developing antibody C which can cause HDFN in subsequent C positive pregnant as well as Hemolytic Transfusion Reactions (HTR) in subsequent C positive red cell transfusion.

Table 4.2 shows the prevalence of Rhesus Du antigen status.In the table, the mean of the Rhesus Du antigen (positive prevalence) was significantly higher (9.20±

3.81) than the negative prevalence of Rhesus Du antigens (0.80± 0.37) at 95% confidence interval. There was no statistical significance in the mean levels of the two Rhesus antigen status, in rhesus (Du) antigen (positive prevalence), p=0.0831 (p>0.05), in rhesus (Du) antigen (negative prevalence), p=0.4327 (p>0.05).

Table 4.3 shows the percentage prevalence of Rhesus Du antigens among the subjects. In the table, 46 out of the 50 samples investigated for Rh Du testing came out positive. This result implies that 92% of the tested samples are positive for Rh Du. The percentage prevalence of negative rhesus Du antigen is 4%. Table 4.4 shows the prevalence of Rhesus C antigen status. In the table, the mean of the Rhesus C antigen (positive prevalence) was significantly higher (2.80± 0.97) than the negative prevalence of Rhesus C antigens (7.20± 3.06) at 95% confidence interval. Also, there was no statistical significance in the mean levels of the two Rhesus C antigen status, in rhesus (C) antigen (positive prevalence), p=0.1084 (p>0.05), in rhesus (C) antigen (negative prevalence), p=0.1258 (p>0.05).

In table 5, 14 samples out of the 50 samples were positive for Rhesus C antigens. This value corresponds to 28%. The 72% was therefore the distribution for negative rhesus C antigens. Our study is also consistent with a previous report by Nwauche and Ejele [28] who studied 65 subject made up of 35 pregnant women and 30 blood donors in the Niger Delta of Nigeria and obtained Rh (C) prevalence of 21.35%. Our observed prevalence is however lower than the prevalence observed in a previous report to determine the presence of clinically significant blood group antigens in the Lao population which indicated a Rh (C) antigen prevalence of 60.43% [29]. Anti-C is a rare antibody and commonly produced in combination with anti-D by (cde/cde pregnant women). It can also be produced in combination with anti-e by R2R2 (cDE/ cDE) individuals. Anti-C seldom causes HDFN and when it does, the disease is usually mild. Anti-C seldom causes HDFN and when it does, the disease is usually mild. Anti-C seldom causes HDFN and when it does, the disease is usually mild. The finding from this study reinforces the advocacy to provide pregnant women and men with child-bearing potential and red cell transfusion of their ABO, Rhesus and Kell phenotypes. This has the potential to reduce the risk of alloimmunization to Rhesus and Kell antigens in donor units that are lacking in the recipient. A previous report [30] suggest matching the red cell phenotype other than ABO and D (C, E, c and K) among the transfusion- dependent patients in an attempt to prevent alloimmunization. Similarly Singer and colleagues (Singer *et al.,* 2000) have reported that patients who received blood matched for Rhesus (C, D, E, c and e) and Kell system from their first transfusion, have relatively lower rate of alloimmunizaton among them. Previous report recommends that all multiply-transfused patients should be phenotyped for the Rh system and that Rh phenotype specific blood is provided in order to limit alloimmunization [31]. Alloantibody C is prevalent among pregnant Nigerian women. In a previous report, Jeremiah and colleagues identified

antibodies in the serum of 17 (3.4%) of their cohort of 500 pregnant women. The specificity of the antibodies was as follows: anti-C 6 (1.2%), anti-E 3 (0.6%), and anti-K 5 (1.0%) [32].

The prevalence obtained in this present study is consistent with a 28%, 21.35% and 60% prevalence obtained respectively in Kano North West- ern Nigeria, Port Harcourt in the Niger Delta of Nigeria and among Lao population. Determination of the distribution of red cell antigen in a population is vital for several reasons; it facilitates the optimum stocking of blood banks with red cells that are negative for clinically significant red cell antigens, it enable the determination of the risk of HDFN and HTR occurring, it allow policy makers to plan for the obstetric and neonatal needs of their population and it allows obstetricians and neonatologist to effectively manage the risk of HDFN. This study re-emphasizes the importance of determining the frequency of red cell blood group phenotypes among individuals of different ethnic backgrounds. Over the last 20-30 years, there has been a change in the demography of most countries due to increased mobility and immigration. The knowledge of the differences in antigen frequency among different populations is also vital in meeting the long-term transfusion need of some transfusion-dependent patients.

Rhesus Du antigen is the most clinical significant red cell antigen in the Rhesus blood group system. Although Rh C, Rh Du and Rh E are closely linked, they are inherited independently of each other. The prevalence of Rh Du negative in the obstetric population of this study was 8%. This is not significantly further from the prevalence range of 4.6% and 5.7% found in the general women population at the Baptist Medical Centre (now Bowen University Teaching Hospital) and the state general hospital, respectively, both in Ogbomoso, by Bakare et al. in the year 2000 [33]. Although a prevalence of 3.3% was found by Bakare and his colleague in the general population in Ogbomoso. The 8% RhDu negative prevalence in the present study is comparable to the 5% from Ibadan, [34] also located in the southwestern Nigeria as is Ogbomoso, but a higher figure than the 4.5% from Enugu, southeastern Nigeria. [33].

Conclusion

This present study indicated a low prevalence in the distribution of Rhesus C (28% positivity) and a high prevalence in the distribution of Rhesus Du (92% positivity) phenotypes among pregnant women in Ogbomoso, Nigeria.

REFERENCES

1. Avent ND, Reid ME. The Rh blood group system: a review. *Blood*. 2000; **95 (2): 375 387.**
2. Okoroiwu IL, Obeagu EI, Christian SG, Elemchukwu Q, Ochei KC. Determination of the haemoglobin, genotype and ABO blood group pattern of some students of Imo State University, Owerri, Nigeria. International Journal of Current Research and Academic Review. 2015 Jan;3(1):20-7.
3. Asemota EA, Njar VE, Aguanah IT, Obeagu EI. Distribution of ABO, Rhesus Blood Group and Helicobacter Pylori Infection among Secondary School Students in Calabar South Local Government, Cross River State, Nigeria. Madonna University journal of

Medicine and Health Sciences.2023;3(1):32-45.
4. Anyiam AF, Obeagu EI, Obi E, Omosigho PO, Irondi EA, Arinze-Anyiam OC, Asiyah MK. ABO blood groups and gestational diabetes among pregnant women attending University of Ilorin Teaching Hospital, Kwara State, Nigeria. Int J Res Rep Hematol. 2022;5:113-21.
5. Eze RI, Obeagu EI, Edet FN. Frequency of Rh Antigen C And c among pregnant women in Sub-Urban area in Eastern Nigeria. Madonna Uni J Med Health Sci. 2021;1(1):19-30.
6. Anyiam AF, Arinze-Anyiam OC, Omosigho PO, Ibrahim M, Irondi EA, Obeagu EI, Obi E. Blood Group, Genotype, Malaria, Blood Pressure and Blood Glucose Screening Among Selected Adults of a Community in Kwara State: Implications to Public Health. Asian Hematology Research Journal. 2022;6(3):9-17.
7. Okoroiwu IL, Obeagu EI, Elemchukwu Q, Ochei KC, Christian GS. Frequency of Transfusion Reactions Following Compatible Cross Matching of Blood: A Study in Owerri Metropolis. International Journal of Current Research and Academic Review. 2015;3(1):155-60.
8. Obeagu EI, Katya MC. A Systematic Review on Physiological Jaundice: Diagnosis And Management Of The Affected Neonates. Madonna University journal of Medicine and Health Sciences. 2022;2(3):25-41.
9. Obeagu EI, Uzoije NU, Afoma I, Ogbodo OR, Onyenweaku FC. Malaria, ABO Blood Group and Haemoglobin Genotypes in Michael Okpara University of Agriculture, Umudike, Abia State, nigeria. Pharmanest. 2013; 4 (5)::1110-1113.
10. Mercy O, Ifeanyi OE, Vincent CC, Prayer N. Association of ABO blood group with HIV infection. J Infect Dis Med Microbiol. 2020;1(1):1-7.
11. Khan MS, Subhan F, Tahir F, Kazi BM, Dil AS. Sultan S. Prevalence of blood groups and Rh factor in Bannu region NWFP (Pakistan). *Pak J Med Res*. **2004; 43 (1): 8!10.**
12. Daniels GL. Human Blood Groups. *2nd edItion.* Blackwell Science. 2002.
13. Reid ME et al. Summary of the clinical significance of blood group allo antibodies. *Semin Hematol*. 2000; 37:197**! 216**.
14. Urbaniak SJ, Greiss MA. RhDhaemolytic disease of the fetus and the newborn. *Blood Review*.2000; **14:44–61**.
15. Hackney DN, Knudtson EJ, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-c isoimmunization. *Obstetrics And Gynecology*. 2004; **103:24–30**.
16. Joy SD, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-E alloimmunization. *Obstetrics and Gynecology. 2005;*.**105:24–8.**
17. Bowman J. (hirty-five years of Rh prophylaxis. *Transfusion*. 2003; **43 (12) 1661–1666.**
18. Zipursky VK, Bhutan. Impact of Rhesus disease on the global problem of bilirubin-induced neuroligic dysfunction, Semin Fetal Neonatal Med. 2015; 20(1) **(2-5.**
19. Zipursky VK, Bhutani. Rhesus disease: a major public health problem, Lancet. 2015; 386-651
20. Zipursky VK, Paul. The global burden of Rh disease, Arch Dis. Child Fetal Neonatal Ed **. 2011; 96(2) f85-5**

21. Pilgrim H, Lloyd-Jones M, Rees A. Routine antenatal anti-D prophylaxis for Rh D negative women: A systematic review and economic evaluation. Health Technology. Assessment.2009; **13**:1-126
22. Turner RM, Lloyd-Jones M, Anumba DO et al. Routine antenatal anti-D prophylaxis in women who are Rh(D) negative: meta-analyses adjusted for differences in study design and quality" *PLoS One*. *2012;* **7 (2) e30711.**
23. McBain RD, Crowther CA, Middleton P. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation". *Cochrane Database Syst. Rev*.2015; **9 (2015) Cd000020.**
24. Fung KF, Eason E. No. 133-prevention of Rh alloimmunization. *J. Obstet. Gynaecol.* Can. **2018; 40 (1) e1–e10.**
25. Dean L. Hemolytic Disease of the Newborn. Blood Groups and Red Cell Antigens. *Bethesda National Center for Biotechnology Information*. 2005.
26. Wagner FF, Flegel WA. RHCE represents the ancestral RH position, while RHD is the duplicated gene'. *Blood*. 2002; **99: 2272–3**.
27. Klein HG, Anstee DJ. The Rh blood group system (and LW). In: Klein. 2005.
28. Nwauche CA, Ejele OA. A Survey of the Status of Common Rhesus Phenotypes in Port Harcourt. *Highland Medical Research Journal*. 2004; **2(1): 22-27**.
29. Keokhamphoui C, Urwijitaroon Y, Kongphaly D, Tham- mavong T. Blood group antigen distribution in Lao blood donors''. *Immunohematology. 2012;* **28(4): 132-136**.
30. Schonewille H, Haak HL, van Ziji AM. Alloimmunization after blood transfusion in patients with hematologic and oncologic diseases. *Transfusion*. 1999; **39(7): 763–771**.
31. Rabeya Y, Suria AA, NurasyikinY, Chooi FL.Incidence of Red Cell Alloantibody among the Transfusion Recipients of Universiti Kebangsaan Malaysia Medical Centre. *Indian Journal of Hematology and Blood Transfusion*. 2013; **29(2): 65–70**.
32. Jeremiah Z.A., Mordi A., Buseri F.I. and Adias T.C. (2011). ''Frequencies of maternal red blood cell alloantibodies in Port Harcourt, Nigeria''. *Asian Journal of Transfusion Sci.* **5(1): 39-41**.
33. Bakare A. Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomoso, South-West Nigeria. *Africa Journal of Biotechnology*.2006; 5:224**-229.**
34. Onwuhafua PI, Adze J. Pregnancy in rhesus negative women in Kaduna, northern Nigeria'. *Tropical Journal of Obstetrics and Gynaecology. 2004;* **21:21-3**.