Original Article

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Quick Response Code:

Website: www.jahjournal.org

DOI: 10.4103/joah.joah_94_22

Immune Erythrocyte Alloantibodies among Pregnant Women Attending an Antenatal Clinic in a Tertiary Health Facility, Benin City, Nigeria

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Submitted: 23-Oct-2022
Revised: 24-Nov-2022
Accepted: 30-Dec-2022
Published: 17-Feb-2023

Abstract:

BACKGROUND: Maternal alloimmunization is associated with adverse outcomes such as hemolytic disease of the fetus and newborn. At-risk pregnant women include those with previous multiple gestations or multiple blood transfusions. This study aimed to determine the proportions and specificities of irregular maternal alloantibodies among antenatal attendees at a federal teaching hospital in Nigeria. An understanding of the pattern of alloimmunization, associated morbidities, and attendant risk factors will guide improved antenatal/perinatal health planning.

MATERIALS AND METHODS: A hospital-based, cross-sectional survey was conducted among 150 pregnant women. Data on parity, transfusion history, and other clinical details were obtained with an interviewer administered questionnaire. ABO/Rh D blood groups and hemoglobin phenotypes were retrieved from their antenatal records and confirmed during the study. Alloantibody screening and identification and other serological tests were subsequently performed. Association of independent parameters with other variables was tested using Chi-square analysis or Fisher's exact as appropriate. Level of statistical significance was set at 5% confidence ($P = 0.05$).

RESULTS: Most of the participants (60%) were in their third trimester, while 9.3% were in first trimester of pregnancy. Ninety-one percent of the participants (90.7%) were blood transfusion naïve. Seven of the participants (4.7%) had positive alloantibody screens, of which two (1.33%) were clinically significant maternal alloantibodies (Anti-D and Anti-Lu^b). No statistically significant association was observed between alloimmunization and variables such as gestational age, parity, hemoglobin phenotype, previous blood transfusions, and Rh D negativity.

CONCLUSIONS: The authors recommend routine alloantibody screening for at risk pregnancies.

Keywords:

Alloimmunization, antenatal, atypical antibodies, Benin City, erythrocyte, irregular alloantibodies, Nigeria, pregnancy

Introduction

Alloimmunization refers to an immune disorder caused by incompatibility between recipient and donor antigens.[1] Alloantibodies of immune-hematologic significance include those formed against foreign red cell antigens, human leucocyte antigens, or human platelet antigens.[2,3] Specific antibodies recognize and interact with specific antigens through their antigenic determinants (epitopes) in a lock and key model.[1,4] Antigen–antibody interactions are affected by the class of the antibody, antigen–antibody ratio, reacting temperature, molecular size of the antigen, pH, ionic strength, and presence of potentiators.[4,5]

How to cite this article: Adewoyin AS, Adeyemi O, Ande AB, Awodu OA. Immune erythrocyte alloantibodies among pregnant women attending an antenatal clinic in a tertiary health facility, Benin City, Nigeria. J Appl Hematol 2023;14:1-6.

In terms of development, red cell alloantibodies may be categorized as naturally occurring or acquired. Naturally occurring alloantibodies such as Anti-A and Anti-B are nonimmune and develop within the first 6 months of life following exposure to ABH-like substances. Antibodies of the ABO, P, and Lewis blood group systems are naturally occurring.[1] Immune alloantibodies, on the other hand, develop as a result of exposure to foreign red cell antigens. Antibodies belonging to other blood group systems such as Rh, Kell, Kidd, Duffy, and MNSs are acquired.[1,5] Their clinical significance relates to the ability of the antibodies to cause *in vivo* destruction of the antigen bearing cells. In the event of red cell alloimmunization, potential complications include hemolytic transfusion reactions (HTRs) and hemolytic disease of the fetus and newborn (HDFN).[1,6]

Maternal erythrocyte alloimmunization is associated with adverse pregnancy outcomes particularly HDFN.[7,8] Limited data exist on the burden of HDFN in Nigeria. Local studies have shown fetomaternal blood group incompatibility as a significant risk factor for neonatal jaundice (NNJ) and HDFN.[9-11] Without appropriate intervention such as intrauterine fetal transfusion, up to 50% of HDFN results in fetal death or severe brain injury.[12,13] The general paucity of data on the burden of posttransfusion and pregnancy related alloimmunization from the African perspective is due to poor hemovigilance.[6] Some local reports show alloimmunization rates of 3.4%–4.8% among pregnant Nigerians.[9,14,15] In some parts of India, the prevalence of unexpected maternal alloantibodies vary between 1.1 and 1.5%.[16-19] A population-based study in Netherlands show positive antibody screens of 1.25% among pregnant women.[20] In Sweden and Canada, the prevalence of maternal alloantibodies was 0.4 and 0.36%, respectively.[21,22]

Lower alloimmunisation rates are reported in developed countries compared to developing countries. Continual vigilance and alloantibody screening is central to managing the associated antenatal/perinatal risks. This study therefore was aimed at determining the proportion and specificities of atypical maternal alloantibodies among antenatal attendees at University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The frequency of Rh D negativity was determined, as well as possible attendant risk factors associated with alloimmunization during pregnancy and childbirth.

Materials and Methods

A hospital-based, analytic, cross-sectional study was performed among pregnant women attending Antenatal Clinic of UBTH between May 2015 and August 2015. Sample size of 70 was calculated using the formula ($n = (z^2pq)/d^2$) for cross-sectional survey based on reported prevalence of 4.8% in Port-Harcourt.[14] One hundred and fifty (150) participants were recruited into the study using nonrandom, convenience sampling. Pregnant women that received any blood components in the preceding 3 months were excluded to prevent false reactions from exogenous antibodies. Furthermore, pregnant woman that had received passive immunization with Anti-D IgG or intravenous immunoglobulin in the preceding 3 months were excluded to prevent false negative reactions. Ethical approval (study protocol number: ADM/E22/A/VOL.VII/1094) was obtained from UBTH Research and Ethics Committee prior to commencement of the study. A written informed consent was obtained from each study participant. Each participant was interviewed with a pretested, structured, interviewer-administered questionnaire to obtain and document relevant biodata and clinical data. Hemoglobin phenotypes, ABO, and Rh (D) blood groups of the study participants were retrieved from their antenatal records and confirmed during the study. Participant's sera were studied for alloantibodies and its specificities using commercially sourced panel of cells. Screening cells were R1R1, R2R2, and rr cells.

The identification panel (10 cell panel) had known antigram containing Rh-hr (D, C, E, c, e, f, V, Cw), Kell (K, k, Kp^a, Kpb, Js^a, Js^b), Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), Lewis (Le^a, Le^b), MNS (M, N, S, s), P1, Lutheran (Lu^a, Lu^b), and additional antigens (Xg^a, Wr^a). Reagent Ig G sensitized red cells were locally prepared.

All assays were done according to standard operating procedures.[23] All reagents were stored according to manufacturer's instructions. Quality control measures to ensure accuracy of antibody screening and identification test include: The screening cells were 2%–5% suspension of 3 vials of typed Blood Group O single-donor red cells. Screening cells displayed homozygous expression of the major blood group antigens. Negative antihuman globulin (AHG) tests were controlled with check cells. Check cells were IgG sensitized (Coombs positive) red cells. 1 volume of reagent red cells was added to each negative test, mixed, and incubated for 1 min at 20°C. The mixture was centrifuged and read. Any negative indirect antiglobulin test that did not show a positive result after addition of check cells was considered invalid and repeated. A direct antiglobulin test control was run for each batch of tests. All centrifugations were carried out at 1000 g for 10 s. Before reading each test after centrifugation, the tube was shaken gently to dislodge the red cell button from the bottom of the tube. All test results were read and interpreted immediately after centrifugation because delay may cause dissociation of antigen–antibody complexes resulting in weak positive or false negative reactions. Reagents cells were stored at

2–8°C when not in use. Potency of AHG reagents were confirmed with Coombs-positive and Coombs-negative cells before each assay. Optimal reacting conditions for antigen–antibody interaction were ensured. The temperature of the water-bath was quality controlled with an external thermometer. Control reagent cells were used on each analytical run to ensure optimal sensitivity, specificity and speed of the reagents used.

Data obtained from questionnaires and results of sample analysis were analyzed using Statistical Package for the Social Sciences (SPSS) version 16 (SPSS Inc., Chicago, Illinois, USA). All descriptive data were analyzed and presented in frequency tables and charts. Level of significance of possible clinical associations/risk factors between alloimmunized and nonimmunized groups was tested using Chi-square analysis or Fisher's exact test where appropriate. Probability score of <5% ($P < 0.05$) was considered statistically significant.

Results

The mean age ± standard error of mean (SEM) of the mothers in the study population was 31.55 ± 0.38 years [Table 1]. The mean gestational age ± SEM at the time they were recruited into the study was 28.21 ± 0.69 years. Most of the participants (60%) were in their third trimester, while 9.3% were in first trimester of pregnancy [Table 1]. Most of the participants (80%) were multigravid, with a mean parity of 2.36. About 9% have had a previous history of HDFN, NNJ or still birth, while 22.7% have had previous cesarean section [Table 2]. Only two of the participants (1.3%) have had passive immunization with Anti-D (Rhogam) before the study [Table 2].

The observed antigen frequencies of the ABO blood group system were 62%, 20.7%, 16.0%, and 1.3% for the O, B, A, and AB antigens, respectively [Table 3]. Most of the participants (95.3%) tested positive for the Rh D antigen. The most prevalent hemoglobin phenotype was AA (77.7%), followed by AS (20.7%), SS (1.3%), and SC (0.7%). About 90% of the participants were blood transfusion naïve.

Seven of the subjects (4.7%) tested positive for unexpected maternal alloantibodies [Table 4]. Five maternal alloantibodies were identified in 5 subjects, 2 were unidentified [Table 5]. Two subjects (1.33%) had clinically significant maternal alloantibody (Anti-D and Anti-Lu^b).

Association between the incidence of alloimmunization and potential risk factors such as gestational age, parity, hemoglobin phenotype, previous blood transfusions, and Rh D negativity were tested [Table 5]. No statistically significant relationship was observed in all groups [Table 5].

Table 1: Obstetric variables of the study participants

Variables	Frequency (%)
Maternal age (years)	
16-25	13 (8.7)
26-35	107 (71.3)
36-45	29 (19.3)
>45	1 (0.7)
Mean±SEM, median, minimum-maximum	31.55±0.38, 31, 19-49
Gestational age (weeks)	
First trimester	14 (9.3)
Second trimester	46 (30.7)
Third trimester	90 (60.0)
Mean±SEM, median, minimum-maximum	28.21±0.69, 30, 11-40
Parity	
Primigravida	30 (20.0)
Multigravida	120 (80.0)
Mean±SEM, median, minimum-maximum	2.36±0.17, 2, 0-10
Previous abortions/miscarriages	
None	74 (49.3)
1-2	55 (36.7)
2 or more	21 (14.0)
Mean±SEM, median, minimum-maximum	1.03±0.11, 1, 0-6

n (%)=150 (100). SEM=Standard error of mean

Table 2: Obstetric history of the study participants

Variables	Frequency (%)
Previous HDFN/NNJ/SB	
Yes	13 (8.7)
No	137 (91.3)
Previous CS	
Yes	34 (22.7)
No	116 (77.3)
Rhogam use	
Yes	2 (1.3)
No	148 (98.7)
Rh D negative and multiparous	
Yes	4 (2.7)
No	146 (97.3)

n (%)=150 (100). HDFN=Hemolytic disease of the fetus and newborn; NNJ=Neonatal jaundice; SB=Still birth; CS=Cesarean section

Discussion

The proportion of Rh D-negative women in the study population was 4.7%. This is consistent with findings from other parts of Nigeria. In a 5-year retrospective survey among 6306 antenatal women in Enugu, Okeke *et al*. reported Rh D negativity of 4.5%.[24] In Nguru, Yobe, Babadoko *et al*. observed Rh D negativity of 4.6% among a cohort of 5519 pregnant women in a study.[25] A slightly higher prevalence of 7.1% was reported among pregnant women in Sokoto, North-West Nigeria.[26] This is noteworthy considering differences in geographical location and influx of immigrants into the Northern parts of Nigeria from the bordering nations.

Table 3: ABO/Rh D blood groups, blood transfusion and alloimmunization status of the participants

Variables	Frequency (%)
ABO blood group	
O	91 (62.0)
B	31 (20.7)
A	24 (16.0)
AB	2 (1.3)
Rh D blood group	
D positive	143 (95.3)
D negative	7 (4.7)
Previous blood transfusion	
Yes	14 (9.3)
No	136 (90.7)
Alloimmunization status	
Positive	7 (4.7)
Negative	143 (95.3)

n (%)=150 (100)

Table 4: Details of alloimmunization in the study participants

Patients	Alloantibod (ies)	Details
Patient 1	Single: Anti-Le^b	31-year-old secondigravida at EGA 37 weeks without previous blood transfusion
Patient 2	Single: Anti-Le^a	36-year-old primigravida at EGA 19 weeks without previous blood transfusion
Patient 3	Single: Anti-Le^a	22-year-old multigravida at EGA 31 weeks, nil previous blood transfusion
Patient 4	Single: Anti-D	41-year-old multigravida at EGA 26 weeks, nil previous blood transfusion; Rh D negative
Patient 5	Single: Anti-Lu^b	30-year-old primigravida at EGA 32 weeks, no previous blood transfusion
Patient 6	Unidentified*	26-year-old primigravida at EGA 19 weeks, no previous transfusion
Patient 7	Unidentified*	29-year-old primigravida at EGA 23 weeks, past history of allogeneic blood transfusion

*Unidentified alloantibodies. 7 of 150 participants (4.7%) were alloimmunized. Five maternal alloantibodies were identified in 5 subjects, 2 were unidentifiable. Two (1.33%) participants had clinically significant maternal alloantibody (Anti-D and Anti-Lu^b). EGA=Estimated gestational age

Among the 7 (4.7%) Rh D-negative women in our study, only 2 of them had anti D postnatal prophylaxis. One of the 7 women with positive antibody screen, who was Rh D-negative had anti-D, a clinically significant alloantibody in her serum. It is pertinent to note that the woman had several second trimester miscarriages. Another clinically significant antibody, Anti-Lu^b, was observed in the serum of another woman with a parity of zero and no previous blood transfusion. Anti-Lu^b has the potential to cause mild HDFN and HTR.[4,27] As such, Lu^b-negative blood units should be transfused to affected individuals. Anti-Lewis antibodies were observed in three other women. Anti-Le^a and Anti-Le^b are common in pregnancy, may be naturally occurring, and are not clinically significant (are not implicated in HDFN).[4,27]

In general, antibodies with known specificities that have been implicated in HDFN/HTR and those that react at 37°C are considered clinically significant.[1,27] In index study, the implicating alloantibodies could not be identified in two of the 7 women with positive antibody screens. Although the antibody(ies) reacted at warm temperature (37°C), it was difficult to make conclusion regarding their clinical significance (their potential for *in vivo* hemolysis). Additional panel of identification cells and immune-hematology techniques will be required for their identification. The commercially sourced reagent red cells used for the experiments were not indigenously produced, hence variations in antigen distribution may account for the unidentified antibodies.

Transfusion of alloimmunized recipients requires use of antigen-negative blood units. Sourcing for antigen-negative blood units may be quite laborious for high prevalence antigens or patients with multiple or rare alloantibodies. No case of multiple alloantibody was observed in index study. Take for instance, a pregnant lady who is being managed for multiple alloantibodies (Anti-C, Anti-s, and Anti-E), assuming the antigen frequencies of C, S and E in the population are 0.45, 0.18 and 0.23 respectively, the probability of identifying a blood unit that will be negative for these antigens will be $0.45 \times 0.18 \times 0.23 = 0.0186$. This implies that 1.86 (~2) of every 100 persons that are screened in the population will be antigen negative. As such, identifying one or two compatible donors for such an alloimmunized blood recipient requires specialized testing of at least 100 blood donors in order to identify antigen-negative units. This will pose a financial challenge and is not cost effective in a developing economy. As such, greater emphasis needs to be placed on prevention, prompt identification and management of erythrocyte alloimmunization.

Theoretically, the potential risk of alloimmunization following fetomaternal (red cell antigen) mismatch increases with advancing gestation as a result of fetomaternal hemorrhage (FMH).[28] However, in this study, women with positive antibody screens were predominantly in their second and third trimesters. No statistically significant difference was observed in the rate of alloimmunization per trimester ($P = 0.256$). Similarly, increasing parity confers a higher risk of alloimmunization through exposure to paternally acquired fetal antigens in antigen-negative mothers.[28,29] Again, there was no statistically significant difference between primigravids and multigravids women ($P = 0.575$). This may be due to the relatively small sample size. Larger, multicenter studies will be necessary to corroborate these findings.

Fetomaternal hemorrhage has been demonstrated to occur in as much as 75% of all pregnancies. The risk

Table 5: Association between alloimmunization and other variable

Variables	Alloimmunization		*P*
	Positive	Negative	
Gestation age			
First trimester	0	14	0.256
Second trimester	4	42	
Third trimester	3	87	
Parity			
Primigravida	1	29	0.575
Multigravida	6	114	
Hemoglobin phenotype			
Non-SCD	7	140	0.866
SCD	0	3	
Blood transfusion			
Yes	1	13	0.504
No	6	130	
Rh D antigen			
D positive	6	137	0.289
D negative	1	6	
Rh D negativity + multiparity			
Yes	1	3	0.176
No	6	140	

n (%)=150 (100). SCD=Sickle cell disease

of FMH increases with advancing gestational age. Therefore, the risk of maternal alloimmunization increases with gestational age. In this study, none of the women in their first trimester was alloimmunized. However, 4 out of 46 women in their second trimester and 3 out of 90 women in their third trimester were alloimmunized. No statistically significant difference was observed in their alloimmunization status across the three trimester groups. This is possible due to unequal proportions of participants across the trimester groups and a relatively small sample size. Maternal age is not directly related to alloimmunization risk. However, there is a general tendency for a higher parity with age.

Three of the study participants (2%) had sickle cell disease (SCD). None of the SCD participants had a positive alloantibody screen. However, SCD patients are at a high risk of clinically significant erythrocyte alloimmunization due to the high rate of blood transfusion.[30,31] Earlier studies among Nigerian SCD patients revealed alloimmunization rate of 7.3%–9.9%, compared to 0% and 4.7% among pregnant SCD and the entire pregnant women in this study respectively.[31,32] The zero prevalence of unexpected alloantibodies among the three pregnant SCD patient despite their positive transfusion history could not be explained. Perhaps, this is related to the insignificant proportion (2%) of SCD women recruited in the study. Larger comparative studies will invariably give a better picture of the risk or association between SCD and maternal alloimmunization. The lower overall prevalence of alloimmunization in pregnant women compared to 7.3-9.9% in SCD patients from the other Nigerian studies may be related to the lower rate of blood transfusion.[31,32] The higher burden of alloimmunization in SCD is related to donor factors such as disparate distribution of donor-recipient red cell antigens and host factors such as increased systemic inflammation and possible immune dysregulation in SCD.[33] In this study, about 9% of the participants have had previous transfusions, compared to a transfusion rate of 36.7%–74.5% in Nigerian sickle cell population.[30,31] Previous blood transfusions have been shown to be an important cause of alloimmunization other than anti-D.[34] Although SCD portends a higher risk of alloimmunization compared to the general population, none of the pregnant SCD women in this study were alloimmunized. No significant difference was observed in the rates of alloimmunization between pregnant SCD and non-SCD women in this study ($P = 0.866$). This may be related to an insignificant proportion (2%) of SCD participants in the cross section, weakening the possible conclusion from this statistical comparison. Similarly, no statistically significant differences were observed between blood transfusion naïve and transfusion experienced cases ($P = 0.504$). In developed nations such as UK, prevalence of Rhesus isoimmunization among pregnant women is much reduced probably due to the routine antenatal use of Anti-D and reduction in family size over the last few decades.[35]

It is important to continually evaluate the burden of alloimmunization in our patient groups, and develop strategies to reduce its incidence. The weakness of the study included unavailability of additional panel of cells to resolve the specificities of unidentified alloantibodies to resolve the specificities of unidentified alloantibodies with possible use of enzyme potentiators. Although clinically significant maternal alloimmunization still occur in the study population, it was found not be related to gestational age, parity, hemoglobin phenotypes, previous blood transfusions, and Rh D negativity. We recommend institutional protocol and national policies should be developed and adopted for detection, prevention and management of maternal alloimmunization by relevant stakeholders. All Rh D-negative mothers should be registered early in a secondary or tertiary health facility with capacity for screening, prevention and management of Rh D alloimmunization. Institutional blood banking service should also be upgraded to include routine alloantibody screening for unexpected maternal antibodies.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Contreras M, Daniels G. Red cell immunohaematology: An introduction. In: Hoffbrand AV, Catovsky D, *et al*., editors. Postgraduate Haematology. 6th ed. Ch. 14. West Sussex: Wiley-Blackwell; 2011. p. 226-43.
2. Birhaneselassie M. Immunohaematology. In: Birhaneselassie M, editor. The Principles of Antigens and Antibodies. Ch. 2. Ethiopia: The Carter Center; 2004. p. 11-9.
3. Williams TM. HLA antigens and alleles. In: Simon TL, *et al*., editors. Rossi's Principles of Transfusion Medicine. Ch. 57. West Sussex: Wiley-Blackwell; 2009. p. 887-97.
4. Bray RA, Pollack MS, Gebel HM. HLA system. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical Manual. 18th ed., Ch. 19. Bethesda: American Association of Blood Banks, Bethesda; 2014. p. 475-98.
5. Judd WJ. Red cell immunology and compatibility testing. In: Simon TL, McCullough J, Snyder EL, Solheim BG, Strauss RG, editors. Rossi's Principles of Transfusion Medicine. Ch. 5. West Sussex: Wiley-Blackwell; 2009. p. 69-88.
6. Natukunda B, Brand A, Schonewille H. Red blood cell alloimmunization from an African perspective. Curr Opin Hematol 2010;17:565-70.
7. Moise KJ Jr. Management of rhesus alloimmunization in pregnancy. Obstet Gynecol 2002;100:600-11.
8. Nardozza LM, Camano L, Moron AF, Chinen PA, Torloni MR, Cordioli E, *et al*. Perinatal mortality in Rh alloimmunized patients. Eur J Obstet Gynecol Reprod Biol 2007;132:159-62.
9. Chinwe AA, John-Kennedy N, Constance N, Hope O. Maternal alloimmunization as a risk factor of haemolytic disease of the foetus and newborn in Owerri Metropolis, Nigeria. Sci J Pure Appl Sci 2013;2:341-7.
10. Ayede AI, Akingbola TS. Pattern, indications and review of complications of neonatal blood transfusion in Ibadan, Southwest Nigeria. Ann Ib Postgrad Med 2011;9:30-6.
11. Ogunlesi TA, Ogunfowora OB, Ogundeyi MM, Ayeni VA. Jaundice among hospitalized Newborn Infants in Sagamu: Observations on aetiology and clinical course. Niger J Paed 2009;36:72-9.
12. Illanes S, Jensen R. Clinical management of haemolytic disease of the newborn and foetus. In: Silverberg DS, editor. Anemia. 1th ed., Ch. 15. Rijeka, Croatia: Intechopen publishers; 2012. p. 247-58.
13. Zipursky A, Paul VK. The global burden of Rh disease. Arch Dis Child Fetal Neonatal Ed 2011;96:F84-5.
14. Jeremiah ZA, Mordi A, Buseri FI, Adias TC. Frequencies of maternal red blood cell alloantibodies in Port Harcourt, Nigeria. Asian J Transfus Sci 2011;5:39-41.
15. Jeremiah ZA, Mordi A. Evaluation of the clinical utility of maternal alloantibody screening as a surrogate to antiglobulin crossmatch procedures in resource limited settings. Int J Blood Transfus Immunohematol 2011;1:16.
16. Suresh B, SreedharBabu KV, Arun R, Jothibai DS, Bharathi T. Prevalence of unexpected antibodies in the antenatal women attending the Government Maternity Hospital, Tirupati. J Clin Sci Res 2015;4:22-30.
17. Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. Blood Transfus 2011;9:388-93.
18. Devi SA, Alwar VA, Sitalakshmi S, Rameshkumar K, Mhaskar R. Red blood cell antibody screening in pregnancy. Asian J Transfus Sci 2011;5:56.
19. Varghese J, Chacko MP, Rajaiah M, Daniel D. Red cell alloimmunization among antenatal women attending a tertiary care hospital in south India. Indian J Med Res 2013;138:68-71.
20. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: A population study in the Netherlands. Transfusion 2008;48:941-52.
21. Gottvall T, Filbey D. Alloimmunization in pregnancy during the years 1992-2005 in the central west region of Sweden. Acta Obstet Gynecol Scand 2008;87:843-8.
22. Zwingerman R, Jain V, Hannon J, Zwingerman N, Clarke G. Alloimmune red blood cell antibodies: Prevalence and pathogenicity in a Canadian prenatal population. J Obstet Gynaecol Can 2015;37:784-90.
23. Regan F. Blood cell antigens and antibodies: Erythrocytes, platelets and granulocytes. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Haematology. 11th ed., Ch. 21. London: Elsevier Churchill Livingstone Publishers; 2012. p. 483-517.
24. Okeke TC, Ocheni S, Nwagha UI, Ibegbulam OG. The prevalence of Rhesus negativity among pregnant women in Enugu, Southeast Nigeria. Niger J Clin Pract 2012;15:400-2.
25. Babadoko AA, Takai IU, Kawuwa MB. Distribution of ABO, Rh D blood groups and haemoglobin phenotypes among antenatal clinic attendees in Federal Medical Centre, Nguru, Nigeria. Borno Med J 2014;11:86-91.
26. Erhabor O, Adamu KS, Yakubu A, Shehu CE, Hassan M, Singh S. Rh (D) phenotype among pregnant women in Sokoto, North West Nigeria. Implications on haemolytic disease of the newborn and haemolytic transfusion reaction. Health Sci Res 2014;1:19-24.
27. Reid ME, Calhoun L, Petz LD. Erythrocyte antigens and antibodies. In: Lichtman MA, Kipps TJ, *et al*., editors. William's Haematology. 8th ed. New York: McGraw-Hills Publishers; 2010. p. 2776-800.
28. Ramasethu J, Luban NL. Allo-immune Haemolytic disease of newborn. In: Lichtman MA, Kipps TJ, *et al*., editors. William's Haematology. 8th ed., Ch. 54. New York: McGraw Hill; 2010. p. 985-1005.
29. Klein H, Anstee D. Haemolytic disease of the foetus and newborn. In: Klein H, Anstee D, editors. Mollison's Blood Transfusion in Clinical Medicine. 11th ed. London: Blackwell Science; 2005. p. 496-545.
30. Otaigbe B. Prevalence of blood transfusion in sickle cell anaemia patients in South South Nigeria: A two-year experience. Int J Med Sci Res 2013;1:13-8.
31. Adewoyin AS. Erythrocyte transfusion and allo-immunisation patterns among sickle cell disease patients, Benin City, Nigeria. Br J Med Med Res 2016;11:1-8.
32. Ugwu NI, Awodu OA, Bazuaye GN, Okoye AE. Red cell alloimmunization in multi-transfused patients with sickle cell anemia in Benin City, Nigeria. Niger J Clin Pract 2015;18:522-6.
33. Chou ST, Liem RI, Thompson AA. Challenges of alloimmunisation in patients with haemoglobinopathies. Br J Haematol 2012;159:394-404.
34. Koelewijn JM, Vrijkotte TG, de Haas M, van der Schoot CE, Bonsel GJ. Risk factors for the presence of non-rhesus D red blood cell antibodies in pregnancy. BJOG 2009;116:655-64.
35. Bowman J. Thirty-five years of Rh prophylaxis. Transfusion 2003;43:1661-6.