We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Distribution of Clinically Relevant Blood Group Antigens among Nigerians and the Management of Rhesus D Negative Pregnancies: Implications for Haemolytic Disease of the Foetus and Newborn and Haemolytic Transfusion Reactions

Erhabor Osaro, Erhabor Tosan, Adias Teddy Charles and Iwueke Ikechukwu Polycarp

Abstract

The ABO and Rhesus blood group systems are the most clinically relevant blood group systems from haemolytic disease of the foetus and newborn (HDFN) and haemolytic transfusion reaction (HTR) perspectives. Other clinically relevant blood group systems include the Kell, Duffy, Kidd and MNSs blood group systems. The clinical relevance of a blood group system depends on the ability of antibodies of the system to cause HDFN and HTR. This chapter discusses the distribution of ABO, Rhesus and other clinically relevant red cell antigens among Nigerians and implications for HDFN and HTR. There are several challenges associated with the management of Rhesus negative pregnancies, pregnancies associated with clinically significant alloantibodies, implementation of policy on routine antenatal anti-D prophylaxis (RAADP), management of Rhesus negative women that require termination of pregnancy (TOP), provision of antigen negative blood for certain patient groups and the management of pregnant and non-pregnant patients with clinically significant alloantibodies. This chapter highlights the need for Nigeria and other African countries to implement best practices to optimize the care offered to pregnant women as well as patients in whom red cell transfusion is indicated.

Keywords: clinically relevant blood group antigens, Nigerians, rhesus D negative pregnancies, haemolytic disease of the foetus and newborn, haemolytic transfusion reaction

1. Introduction

1.1 History of Nigeria

Nigeria often referred to the giant of Africa is the largest Black nation in Africa. It is a country located in West Africa bordered by Cameroon in the East, Benin Republic in the West, Niger Republic to the North and Chad Republic to the North East. It is a federation consisting of 36 states with Abuja the Federal Capital territory as the capital. It is located on the Gulf of Guinea with a total area of 923,768 km^2 (356,669 sq. miles). It is the world's 32nd-largest country. Nigeria got her independence from the British on 1st of October 1960. It has an estimated population of over 200 million people. The United Nations estimated the population in 2016 to be 185,989,640 distributed as 51.7% rural and 48.3% urban with a population density of 167.5 persons per square kilometre. Although the official language is English, there are more than 250 ethnic groups with well over 500 different native languages and diverse cultures. There are 3 major ethnic groups in Nigeria (Hausa/Fulani, Igbo and Yoruba). Nigeria is a religiously diverse nation divided into a predominantly Christian South and Muslim North and a minority groups of traditional African religion. Nigeria is a mixed economy dependent and an emerging market. As at 2014 Nigeria was the fastest growing and largest economy in Africa. She is blessed with abundant natural resources and a developing communication, financial, legal and transportation sector. Nigeria is the 12th largest producer of oil in the world, the 8th largest exporter, has the 10th largest proven reserves and supplies a fifth of its oil to the United States of America. Oil plays a significant role in the Nigerian economy and accounts for 40% of GDP and 80% of all government earnings. Healthcare delivery is the responsibility of the 3 tiers of government (federal, state and local government). There is a number of health-related challenges in Nigeria (HIV/AIDS, malaria, polio, poor access to potable and clean water, lack of proper sanitation system, high infant and maternal mortality rates). Although the recurrent expenditure on health in Nigeria has increased over the years, healthcare delivery and infrastructural endowment remains suboptimal with health tourism on the increase [1]. The Nigerian health care system is continuously faced with a number of challenges; shortage of healthcare workers [2] predominantly due to significant emigration of skilled medical personnel to developed economies of the world (brain drain), suboptimal funding, decaying infrastructure, inter-professional conflict, perennial strike actions by healthcare professionals, lack of political will as well as bureaucratic bottlenecks in public health care delivery in Nigeria [3].

2. UHC and the WHO model lists as governmental obligations to implement before 2030

Nigeria is a party to the United Nation (UN) General Assembly adopted 2030 agenda for Sustainable Development and the UN High-Level Meeting (UN HLM) Universal Health Coverage (UHC) UHC2030. In September 2015, the UN General Assembly adopted the 2030 Agenda for Sustainable Development that included 17 Sustainable Development Goals (SDGs) ensuring that all persons are carried along and that no one is left behind [4]. The agenda laid emphasis a holistic approach aimed at achieving sustainable development for all. The 17 sustainable development goals (SDGs) included; no poverty, zero hunger, good health and well-being, quality education, gender equality, clean water and sanitation, affordable and clean energy, decent work and economic growth, industrial innovation and infrastructure, reduced inequality, sustainable cities and communities, responsible consumption

and production, climate action, life below water, life on land, peace and justice strong institutions and partnerships to achieve goal. On September 23rd 2019, a high-level meeting on universal health coverage held at the United Nations General Assembly. The meeting focussed on Universal Health Coverage: Moving Together to Build a Healthier World (UHC2030) and brought together heads of state, political and health leaders, policy-makers, and universal health coverage champions to advocate for health for all. The meeting agreed on Key Asks from the UHC Movement aimed at accelerating progress toward Universal Health Coverage (UHC) [4]. Among others, UHC2030 advocates that countries should; ensure political leadership beyond health (that all countries to ensure healthy lives and wellbeing for all at all stages as a social contract), leave no one behind (pursue equity in access to quality health services with financial protection), regulate and legislate (create a strong, enabling regulatory and legal environment responsive to people's needs), uphold the quality of care (build high-quality health systems that people and communities trust), invest more and better (increased domestic investment and allocation of more public financing for health through equitable and mandatory resource) and move together (establishing multi-stakeholder mechanisms for engaging the whole of society for a healthier world). Nigeria is a party to the global commitment ensuring the well-being, universal health coverage and access to quality healthcare when and where they need it, without facing financial hardship [5]. There is general expectation among Nigerians for the government to mix word with action by ensuring that all Nigerians in both rural and urban communities have access and can use the promotive, preventive, curative, rehabilitative and palliative health services they require, that is of sufficient quality and affordable [6].

3. Current situation in Nigeria

3.1 Distribution of ABO and other clinically significant blood groups

The human red blood cell membrane is complex and contains a number of clinically relevant blood group antigens, the most relevant being the ABO and the Rhesus blood group antigens. Apart from the ABO and Rhesus blood group systems, thirty-four other blood group systems have been identified as at November 2014 [7]. In addition to the ABO and Rhesus blood group antigens, 364 other red cell antigens have been identified serologically. The clinical relevance of a blood group system depends on the distribution of antigens of the blood group system in the population, ability of antibodies of the blood group system to cause haemolytic transfusion reaction (HTR) and haemolytic disease of the foetus and newborn (HDFN) [8]. The ABO blood group system is one of the most clinically relevant blood group systems [9]. It was first discovered by Karl Landsteiner in 1901 [10]. The ABO blood group system has three main antigens (A, B and H). Four major ABO groups exist (A, B, AB and O). The ABO blood group system is based on the A and B antigens occurring singly as A or B, doubling as AB or the absence of both as O. Individuals who have lack the A or B antigens on their red cells have the group specific antibody in their serum or plasma. Antibodies of the system are predominantly IgM originally thought to be naturally occurring but are now known to occur in the first years of life as a result of sensitization to ABO-like antigen and environmental substances that occur in nature such as bacteria, viruses and food. Evidence has shown that these antibodies are not present at birth and that animals kept in a sterile room from birth do not produce these antibodies. The ABO blood group system is important in transfusion medicine, HDFN and in organ transplant. Transfusion of ABO incompatible unit can cause severe HTR. Similarly, an immune response can also occur

following ABO incompatible organ transplant. ABO blood group incompatibilities between the mother and child does not normally cause HDFN because antibodies of the ABO blood group system are usually of the large molecular weight IgM type, which do not cross the placenta. However, mothers who were previously sensitised (previous incompatible transfusion and pregnancy) can potentially have IgG ABO antibodies that can potentially cause ABO HDFN. The distribution of ABO blood groups varies across the world according to the population. There are also variations in blood type distribution within human subpopulations.

Nigeria is a significantly varied nation in terms of ethnicity. The gene frequency of ABO and Rh blood group varies significantly within the six geopolitical zones in Nigeria [11–18]. Previous studies in most parts of Nigeria indicates that the ABO blood group distribution is in the order O > A > B > AB [19]. Studies from the United States, Mauritania, Morocco, Cameroun, Tunisia, Ethiopia and Iran reported same order (O > A > B > AB) [20–26]. However, study in Madagascar and Guinea reported a contrary trend (O > B > A > AB) [27, 28]. This observation in Nigeria is also at variance with reports from India and Bangladesh where prevalence of B is highest followed by O and the least was AB (B > O > A > AB) [29, 30]. Reports from Turkey and Colombia indicates an order A > O > B > AB [31, 32].

A multi race/ethnic study in the United States reported that blood type O is the most prevalent (46.6%) with White non-Hispanic, Hispanic, Black non-Hispanic, Asian and North American Indian having varying percentage of 45.5, 56.5, 50.2, 39.8 and 54.6 respectively [21]. Other studies in Turkey, Mauritania, Iran, Ethiopia, Colombia, Cameroun, Bangladesh, Madagascar, Morocco, Guinea and Northern India have reported varying percentage in ABO and Rh blood types [20–23, 25–32].

Phenotypic distribution of Rh(D) in Nigeria varies from one part of the country to the other; Kwara (4.5%) [17], Jos Plateau State (4.32) [14], FCT (4.3) [33], Minna (3.3%0) [11], Lagos (3.0, 6, 6.86%) [16, 34, 35], Ogun (6.65 and 2.9%) [36, 37], Osun (6.3%) [38], Oyo (3.3, 6.68, 4.8, 5.89%) [12, 14, 18, 39], Ekiti (4.3%) [40], Akwa Ibom (5.7%) [41], Bayelsa (2%) [42], Delta (1.8%) [43], Benin (6.0%) [13], Rivers (3.2, 7, 8.3%) [44–46], Kano (5.2%) [47], Sokoto (1.55%) [14], Zamfara (1.2%) [48], Enugu (4.49%) [14], Abia (5.3%) [49], Ebonyi (4.2%) [50], Borno (1.92%) [14], Adamawa (4.6%) [51], Yobe (4.6%) [52]. The prevalence of Rhesus negativity varies from one zone to the other; 4.4, 3.1, 6.0, 4.3, 3.9 and 3.1% respectively for the South East, North East, South, South, South West, North Central and North West zone respectively.

The overall average prevalence of Rhesus negativity is 5.1%. The distribution of Rh(D) in Nigeria is in agreement with others parts of the world [20, 23, 25–28, 30, 31]. Blacks have been found to have a lower frequency of Rhesus D negative phenotype (3–5%) [34, 42, 44] compared to the general Caucasian population (15%) [21, 53]. The lower prevalence of Rh(D)− in Nigeria and other developing is important and a blessing in disguise because clinical situations like fetomaternal haemorrhage during the course of pregnancy can give arise through Rh incompatibility and HDFN. In Nigeria and many of these developing countries RAADP and other anti-D HDFN related prevention strategies are not being implemented. **Table 1** shows the prevalence of Rhesus D group among Nigerians based on zones.

The Kell blood group is the third most clinically relevant blood group system after ABO and Rhesus. Individuals without Kell antigens (K_0) who are transfused with Kell positive donor red cells or Kell negative pregnant women exposed to the Kell positive red cells of their baby carry the risk of developing Kell antibody which can cause HDFN [54]. In the developed world all pregnant women and non-pregnant women of child bearing age are transfused with Kell negative blood. Also, patients with other antibodies are transfused with Kell negative and red cells also lacking the antigen to which their alloantibody is specific. This is to prevent them from potentially developing anti-Kell antibody. Kell sensitization is the third

Zone of Nigeria	Percentage of Rhesus negative status	Percentage of Rhesus negative status
South-East	4.4	95.6
South-South	6.0	94
South-West	4.3	95.7
North-East	3.1	96.9
North-West	3.1	96.9
North-Central	3.9	96.1

Table 1.
Prevalence of Rhesus D group among Nigerians based on zones.

most common cause of HDFN after Rh and ABO. Anti-Kell has been shown to cause severe foetal anaemia by suppressing foetal RBC synthesis [55]. HDFN-associated anaemia in Anti-Kell-related HDFN is caused by the ability of anti-K to cause the suppression of foetal production of RBCs [56]. Unlike Rh and ABO, Kell antigens are expressed on the surface of RBC precursors, and anti-K promotes the immune destruction of Kell positive erythroid early progenitor cells by macrophages in the foetal liver rather than only mature foetal RBCs [55]. There are few Kell prevalence studies in Nigeria. Among their cohort of pregnant women in Sokoto, North Western Nigeria, Erhabor and Colleagues [57] obtained a Kell antigen prevalence of 2%. Similarly, Ugboma and Nwauche [58] in Port Harcourt investigated the prevalence of Kell antigen among their patients and reported a Kell antigen prevalence of 2%. The prevalence of Kell antigen among a multi-ethnic cohort of 302 healthy Nigerian individuals indicated a zero prevalence of K antigen [59]. Racial differences seem to exist in Kell blood group antigen distributions [60, 61]. The Nigerian government and governments in other African countries will need to implement this strategy in a bid to reducing Kell-related sensitization and the effect of HDFN.

The prevalence of other clinically significant red cell antigens has been determined among Nigerians. The prevalence of Lewis, Kidd, Duffy, Kell and M blood group antigens among blood donors in Aminu Kano Teaching Hospital, Kano, Nigeria [62] were as follows: Lea: 26.4%, Leb: 15.1%, M: 20.8%, k (cellano): 21.7%. The Duffy (anti Fya, anti Fyb) and Kidd (anti Jka anti Jkb) antigens were not detected among the donors. Out of the 162 pregnant women tested for their Duffy antigens status indicated Fya, Fyb and Fya (a+b+) prevalence of 7 (4.3%), 9 (5.6%) and 1 (0.61%) respectively [63]. Kidd blood group phenotypes were determined among pregnant women in Sokoto, North Western Nigeria [63]. The distribution of Kidd antigens among subjects studied indicated a prevalence of Jka, Jkb and Jk(a+b+) of 8 (4.9%), 13 (8.0%) and 0 (0.0%), respectively. There is need for the phenotyping of donor's blood for clinically significant red cell antigens. There is also the need to routinely screen all pregnant women for alloantibodies to facilitate the selection of antigen negative units for those with clinically significant alloantibodies who require a red cell transfusion. This can potentially optimise the obstetric management of HDFN.

The prevalence of Rh c and e phenotype among 200 pregnant women attending antenatal clinic (ANC) in Usmanu Danfodiyo University Teaching Hospital Sokoto was determined. The prevalence of Rh c was 92% while Rh e was 98.5% [64]. The frequencies of Rh blood group antigens and phenotypes of the Ibibio, Efik, and Ibo ethnic nationalities in Calabar municipality, Nigeria, were determined using standard serologic techniques. Of the 720 Calabar individuals tested, the frequencies of the Rh antigens within the nationalities were c (100%), e (96.38%),

D (96.38%), E (15.22%), and C (3.62%) for the Ibibios; c (100%), e (95.60%), D (96.70%), E (21.98%), and C (0%) for the Efiks; and c (100%), e (94.29%), D (91.43%), E (28.57%), and C (2.86%) for the Ibos. The overall frequencies of the Rh antigens in these 720 individuals were c (100%), e (95.56%), D (94.44%), E (18.89%), and C (2.78%). Forty (5.56%) were found to be D−, while all were found to possess the c antigen. The most frequently occurring Rh phenotype was Dccee, with a frequency of 73.61%. The alternative allele, C, did not appear in homozygous form (CC) in the population tested [65]. Of the 374 pregnant women studied in Port Harcourt, Nigeria, the frequencies of the Rh antigens within the population were D (89.0%), c (82.0%), e (54.0%), C (24.3%), E (20.1%). The frequencies of the Rh antithetical antigens were DD/Dd (91.2%), Cc (19.5%), cc (84.5%), Ee (13.9%), ee (54.3%), CC (25.1%), EE (19.8%) and dd (10.4%). Seven (1.9%) were found to be Rhnull, sixteen (4.3%) were found to be D− or exalted D. Phenotypes without RhD reactivity were -c- (2.9%), -Cc (0.3%), -C- (0.3%), -Ee (0.5%) and -E- (0.3%) [66]. A multi-ethnic cohort of healthy Nigerian individuals were studied. The antigen status of these individuals for Rh was determined. The prevalence of the Rh antigens in the study cohort was found to be: D (92.7%), C (20.5%), c (97.7%), E (19.5%), and e (97.4%). Dce was the most common Rh phenotype (53.3%) [59]. Few countries in sub-Saharan Africa have systematic testing for antigens C, c, E, and e of the Rh and Kell system antigens in the donor and recipient, thereby exposing transfused patients to the risk of developing antibodies that can cause HTR and HDFN. Among 651 blood donors tested in Abidjan for antigens of the Rh blood group system, the antigen frequencies of D, c, e, C, and E were; 92.93, 99.85, 99.85, 21.97, and 13.82% respectively. K antigen is was found in 0.77% of donors [67].

3.2 Universal donor phenomenon: the need to implement a policy of universal haemolysin testing of group O donor units

ABO blood group system is one of the most clinically relevant blood group systems. Individuals above the age of 6 months have clinically significant anti-A and/or anti-B in their serum particularly if they lack the corresponding antigens on their red cells. Whole blood transfusion still thrives in Nigeria despite the advantages of component therapy and challenges associated with whole blood transfusion (development of febrile transfusion reactions, graft-versus-host disease, alloimmunization to leukocyte antigens, immunomodulatory effects, transmission of diseases such as cytomegalovirus, Human T-Lymphotrophic Virus I/II and Epstein Barr (EBV) for which leukocytes may be the vector) [68]. Blood group O donor blood is the most common blood group among Nigerian blood donors [69] and their red cells are commonly used as universal donor units for transfusion to A, B and AB recipient particularly in emergency situations. Blood group O individuals lack the A and B antigens on their red cells and thus are inappropriately called Universal donors. These individuals have anti A and Anti B in the plasma. Due to the absence of A and B on their red cell, their red cells could be given to A, B, and AB individuals. This is quite useful particularly in Nigeria and other developing countries where there is inadequate supply of donor blood. However, some group O individuals have potentially lytic anti-A and lytic anti-B in their plasma that bind to antigens A and B on the surface of erythrocytes in recipients and potentially activate the complement cascade resulting in acute intravascular haemolysis. To avoid this, all blood group O donor units intended for transfusion to non-O patient must be tested for the presence of these anti-A and lytic anti-B haemolysis. Those donors found negative can have their blood given to non-O patients while those that test positive are reserved for group O recipients only. Haemolysin testing to

identify the presence of haemolytic anti-A and anti-B antibodies has emerged as a useful screening in most countries to identify high levels of anti-A and/or anti-B antibodies to prevent HTRs. Routine haemolysis testing of blood group O donor units intended for transfusion to A, B or AB recipient is not routinely carried out in most settings in Nigeria despite the high prevalence of haemolysins observed among Nigerians in previous studies [70–75]. The Nigeria government can learn from evidenced-based best practices in most developed settings [76, 77] and implement a policy to routinely test all group O donor units for haemolysins in other to identify group O donors with high titre of IgG anti A and/or anti B whose blood should be reserved only for transfusion to group O recipient while those that test negative can be transfused to A, B or AB individual as a way to maximising the use of our limited allogeneic stock.

3.3 Screening for clinically significant alloantibodies

Alloantibodies are antibodies produced in a patient as a result of exposure to foreign red cell antigen via transfusion, pregnancy or transplantation. In countries such as Nigeria, there are multiple ethnic groups and racial or genetic heterogeneity among the population. This can often be associated with a wide variation of alloantibodies [78]. Other common factors that facilitate alloantibody formation in the recipient include: the immune competence, the dose of the antigen the recipient is exposed to, the route of exposure and how immunogenic the foreign antigen is [79, 80]. Development of alloantibodies can lead to difficulty in finding compatible blood for transfusion or it can result in severe delayed haemolytic transfusion reaction if the antibody titre is low, undetected, missed and if antigen positive units is transfused [81]. Evidenced-based best practice in the developing world requires that alloantibody testing is carried out as part of pre-transfusion testing of patients who require a red cell transfusion as well as pregnant women presenting to antenatal clinic at booking [82, 83]. The aim of this test is to detect the presence of unexpected red cell antibody in the patient's serum [84, 85]. Once these antibodies are detected during the alloantibody screening, every effort must be made to identify the specificity of the alloantibody by doing a panel test. The aim of identifying the specificity of the alloantibody in a patient that requires a red cell transfusion is to enable the Medical Laboratory or Biomedical Scientist to select antigen negative donor unit for appropriate crossmatch (indirect antiglobulin test) for such patient [86]. The aim of a panel test in the case of a pregnant women coming for antenatal booking is to identify the alloantibody, determine whether the antibody can potentially cause HDFN [87] and to allow the monitoring of the titre or quantification of the antibody every 4 weeks from booking until 28 weeks' gestation and every 2 weeks thereafter until delivery [88]. The obstetrician requires this information to determine to what extent the developing foetus is affected by HDFN, decide whether to monitor the baby for anaemia using Doppler ultrasound, determine whether the baby will require intrauterine transfusion and to make an informed decision to possibly deliver the baby earlier. These evidence-based best practices are not being implemented in many settings in Nigeria. Testing of donor units for other clinically relevant red cell antigens other than ABO and Rhesus D is not routinely carried out. Also, donor units particularly those intended for transfusion to pregnant women and neonates are also not routinely screened for CMV and Hepatitis E virus like it is routinely done in more advanced part of the world. This is a complete failure in stewardship by the Nigerian government and can compromise the transfusion service delivery to pregnant women and patients that require red cell transfusion.

4. Challenges for the future

4.1 Absence of universal access to prophylactic anti-D through the routine antenatal anti-D prophylaxis (RAADP) program

RAADP or routine antenatal anti-D prophylaxis is a recommended treatment option for all Rhesus D negative pregnant women who are not known to be sensitised previously to the RhD antigen. The D antigen is the most immunogenic and most clinically relevant of the Rhesus antigens. A mother who is RhD negative and married to a homozygous or heterozygous D positive husband has a 100 and 50% chance respectively of carrying a D positive baby. During such pregnancies as a result of feto maternal haemorrhage (FMH) following potentially sensitising events [abdominal trauma, abortion or termination of pregnancy, antepartum haemorrhage (APH), amniocentesis, chorionic villus sampling, external cephalic version (ECV) and miscarriages] during pregnancy, small amounts of foetal blood can enter into the maternal circulation. The foetal RhD-positive cells can irreversibly sensitise the mother to produce alloantibody D. The risk of sensitisation occurring is dependent on a number of factors; ABO blood group of the developing foetus (there is a higher risk if there is ABO compatibility between mum and baby), dose of foetal cells entering the maternal circulation, immune competence of the mother and type of pregnancy (risk is significantly higher in the first decreasing in the subsequent pregnancies. The antibody produced is immune IgG antibody that is a low molecular weight antibody and can potentially cross the placenta barrier. The maternal immune anti-D are small molecular weight IgG immunoglobulin and can pass through the placenta in subsequent D positive pregnancy and destroy the foetal red cells with increased production of bilirubin a product of red cell breakdown (HDFN). While in womb the mother manages the bilirubin on behalf of the baby by production of glucoronyl transferase enzyme which break down (conjugate) the bilirubin to water soluble forms that can be excreted in the urine. However, after delivery, the excess bilirubin released from the breakdown of the foetal red cells leads to jaundice. With the baby's liver poorly developed and not producing enough glucoronyl transferase enzyme to conjugate the bilirubin to excretable form, the level can potentially rise above the blood brain barrier and cause kernicterus (permanent brain damage) with attendant neurodevelopmental problems (cerebral palsy, deafness neuromotor and speech delay). Routine antenatal anti-D prophylaxis (RAADP) is a program under which Rhesus D negative non-sensitised pregnant women are universally offered anti-D prophylaxis at 28–34 weeks' gestation with the aim of preventing the sensitization of Rh D-negative women and by extension prevent anti-D HDFN in subsequent pregnancy [89]. The aim of the RAADP programme is the reduction in the incidence of anti-D related HDFN; improve the survival of the children delivered by Rhesus D negative women, reduce the incidence of disability and health-related quality of life of children and mortality in children delivered by Rhesus D negative women who would have developed anti-D related-HDFN if mothers were not offered prophylaxis. The RAADP program is based on offering either two doses of at least 500 IU at 28- and 34-weeks' gestation or a single dose of at least 1500 IU at 28 weeks gestation followed by a further dose of at least 1500 IU within 72 h of delivery of a Rh D positive baby. The dose offered post-delivery is dependent on the result of feto maternal haemorrhage testing result (flow cytometry of Kleihauer testing). Both methods quantify in millilitres the amount of foetal red cells that has entered maternal circulation to facilitate the administration of adequate dose of prophylactic anti-D. As a general rule 1 25 IU of anti-D is administered to clear 1 ml of foetal red cells from maternal circulation. The introduction of anti-D prophylaxis using Rhesus D immunoglobulin

(given intramuscular or intravenous administration) has led to a significant fall in the number of women becoming sensitised and by extension reduced the incidence and severity of this condition. The anti D immunoglobulin used as prophylaxis is prepared from plasma of male Rhesus D negative donors who have high levels of plasma anti-D due to deliberate or intentional immunisation with D positive red cells. Anti D is also administered to Rhesus D negative women following a potentially sensitising events during pregnancy. The anti-D facilitates the clearance of foetal red cells from the maternal circulation to prevents active immunisation, thus preventing the production of alloantibody D. Prior to the availability and widespread use of anti-D prophylaxis for Rhesus negative pregnant women, the incidence of Rh D sensitization among Rhesus D negative women following two deliveries of D positive and ABO-compatible, infants was approximately 16% and haemolytic disease of the foetus and newborn (HDFN) due to immune anti-D was a significant cause of morbidity and mortality [90]. Following the implementation of anti-D prophylaxis, the rate of sensitization has declined significantly to approximately 2%. With the implementation of RAADP by providing anti-D prophylaxis in the 3rd trimester between 28 and 34 weeks gestation, there has been a further remarkable reduction in the sensitisation rate to 0.17–0.28% [91]. Mortality from D-related HDFN related has also declined significantly from 46/100,000 births to 1.6/100,000 births [89]. Evidence from best practice implementation in England and Wales indicates that RAADP has reduced the incidence of sensitisation and hence of HDFN [92]. Conservative estimate indicates that it cost £2–£3.5 million for England and Wales to provide RAADP to all her Rh D-negative pregnant women. The Nigerian government can learn from this evidence-based best practice by implementing RAADP program for her population of Rhesus D negative pregnant population with the hope of reducing sensitization, incidence and severity of anti-D related HDFN and mortality in Nigeria.

4.2 Absence of facility for foetal genotype testing for D, K and other clinically relevant red cell antigens

Rhesus D grouping is relevant for blood donors, transfusion recipients and for women of child bearing age including pregnant women. This is because the Rh blood group antigens particularly D is significantly immunogenic. Rh D negative individuals often lack the D antigens on their red cells and can potentially be sensitised when exposed to D antigen positive red cells during pregnancy and blood transfusion. Such antibodies are often capable of causing a haemolytic transfusion reaction (HTR) and haemolytic disease of the foetus and newborn (HDFN). Since the introduction of prophylactic anti-D and implementation of evidence based best practice of careful management and monitoring of D negative pregnant women for all the potentially sensitising events that occur during pregnancy, the prevalence of HDFN because of Rh D incompatibility between the mother and baby has declined significantly [90]. In order to prevent Rhesus D negative women who are not previously sensitised from developing alloantibody D, these women are offered prophylactic anti-D during pregnancy under the Routine Antenatal Anti-D Prophylaxis program between 28 and 34 weeks' gestation. The half-life of the administered anti-D is 12 weeks. The anti-D prevents the mother from being sensitised by micro foetal maternal haemorrhage that can occur as a result of potentially sensitising events that occur during pregnancy. These women should have a fetomaternal haemorrhage testing following any potentially sensitising events that occur after from 20 weeks' gestation. This test quantifies the amount of foetal red cells that entered the maternal circulation to allow for the administration of adequate dose of anti-D to clear the fotal cells and prevent them from

sensitising the mother. Anti-D (125 IU) is required to remove 1 ml of foetal red cells from maternal circulation. RhD alloimmunisation is still a significant cause of foetal and neonatal morbidity and mortality particularly in Nigeria and other developing countries [93–95] because of absence of evidence-based best practices and non-implementation of Rh D prophylaxis during pregnancy. This often result in a significant number of unfortunate women developing alloantibody D through no fault of theirs but rather as a result of failure in stewardship by the Nigerian government. Also, non-invasive foetal blood group genotyping (DNA) testing of maternal plasma to determine the antigen status of the developing foetus carried by an alloimmunised pregnant women is vital as it provides useful information as to whether a foetus is carrying the group specific antigen and in fact at risk of HDFN. This will help prevent the need for extensive laboratory testing (titration and quantification of antibody every 4 weeks from booking till 28 weeks' gestation and every 2 weeks from 28 weeks to delivery) and clinical monitoring in antigen negative cases. Molecular testing of maternal plasma for foetal DNA can be performed during the second trimester. DNA probes for the most common antigens associated with HDFN are now available [96]. Knowledge of the molecular basis of the blood group systems has facilitated the development of assays for blood group genotyping. Foetal Rhesus D genotyping can potentially tell at 16 weeks' gestation through the analysis of amniotic fluid or through maternal plasma the foetal D genotype of the developing foetus. In previously sensitised alloimmunised pregnant women, knowledge of the foetal D antigen status is beneficial to enable obstetricians optimally manage these women [95]. In non-sensitised Rhesus negative pregnant women, the knowledge of the foetal D antigen status is quite important for several reasons; prevent us from exposing the mother to a prophylactic anti-D (a human blood product that even though significantly virally tested for TTIs) she does not need, allow for the optimal utilisation of the product in patients in whom it is indicated and spare the woman the pain of the product being administered intramuscularly. *RHD* genotyping of foetuses carried by Rh D-negative women using foetal DNA obtained invasively through amniocentesis or chorionic villus sampling used to be critical to the clinical management of these women. Technological advances now allows for accurate determination of foetal *Rh D* genotype using cell-free foetal DNA from maternal blood, thus overcoming the invasive procedures [97]. *Rh D* genotyping are based on polymerase chain reaction using non-invasive blood sample from the mum are quite sensitive with low incidence of false positive result [98, 99]. Although there are cases of D-negative genomes possessing fragments of mutated Rh D genes, the most notable of which is the Rh D pseudogene found in Africans. Rh D genotyping tests have been developed to differentiate these alleles and thus enhance the diagnosis in a multi-ethnic population [100].

Foetal blood group genotype of a developing foetus can also be determined for Rh C, c, E and Kell (K) using cffDNA from maternal plasma [101]. Many European Union countries have suggested the mass application of foetal genotyping for all foetuses carried by D-negative women. This advocacy is based on clear benefit of conserving anti-D stocks and prevention of unnecessary administration of this human-derived blood product that has associated risk [102, 103]. In Denmark and the Netherlands this evidenced-based best practice of foetal *Rh D* testing for all non-sensitised D negative pregnant women have been introduced [104]. Also, non-invasive foetal blood group genotyping (DNA) testing of maternal plasma to determine the antigen status of the developing foetus carried by an already alloimmunised pregnant women is vital as it provides useful information as to whether a foetus is carrying the group specific antigen and in fact at risk of haemolytic disease. This will help prevent the need of extensive laboratory (titration and

quantification of antibody every 4 weeks till 28 weeks' gestation and every 2 weeks from 28 weeks to delivery) and clinical monitoring in antigen-negative cases.

4.3 Absence of facility for intrauterine transfusion for children with HDFN that are severely anaemic in utero

The maternal alloantibody D produced as a result of sensitization of the Rh D negative mother to the Rhesus D positive foetal red cells are low molecular weight IgG antibody. This antibody can potentially cross the placenta barrier into the foetal circulation and can destroy the foetal red cells resulting in anaemia, heart failure, hydrops foetalis (fluid retention and swelling) and intrauterine death. In utero the diagnosis of foetal anaemia used to be made by carrying out ultrasound guided foetal blood sampling for analysis of the foetal haemoglobin and haematocrit or amniocentesis-an invasive ultrasound guided procedure with a significant 2% risk of foetal loss associated with entering the amniotic sac and obtaining amniotic fluid which is analysed for product of haemoglobin breakdown. However, non-invasive diagnosis of foetal anaemia can now be made using non-invasive Doppler ultrasound technology by measuring the middle cerebral artery peak systolic velocities (MCA PSV). The foetal anaemia can be managed with intrauterine blood transfusions. Intrauterine transfusion is associated with a number of risks; foetal bradycardia, foetal death, cord haematoma, haemorrhage from the puncture site, miscarriage, preterm labour and vessel spasm. The blood used for such transfusions must meet certain requirements; gamma irradiated red cells to prevent transfusion-associated graft-versus-host disease, haemoglobin S negative, CMV negative and significantly high PCV (0.70–0.85) [105, 106], unit must be C-, D-, E- and K-, leucocyte depleted to less than 1×10^6 leucocytes per unit, less than 5 days old, free from clinically significant irregular blood group antibodies and negative for high-titre anti-A and anti-B haemolysin. If the mother has other alloantibodies apart from D, it is vital that suitable unit negative for the specific antigens to which those maternal alloantibodies are specific are selected. Once the baby is delivered, intensive phototherapy and IV immunoglobulin along with antiglobulin (DAT) test should be carried out. There should also be continuous monitoring of the haemoglobin, haematocrit and bilirubin level. A positive DAT is diagnostic of HDFN and the baby sample should be sent for elution to identify if the antibody coating the foetal red cell is maternal alloantibody. If the bilirubin is not responsive to intensive phototherapy and continues to rise, the associated neonatal anaemia and hyperbilirubinemia can be managed by carrying our exchange blood transfusion (EBT) to prevent bilirubin encephalopathy by removing a significant number of maternal antibodies-coated foetal red cells and excess bilirubin and replacing it with donor red cells. The red cell product used for EBT must meet certain requirements; group O or ABO compatibility (mum and baby), compatible with any maternal antibody, gamma irradiated, collected in saline, adenine, glucose and mannitol (SAGM), fresh less than 5 days old, have high haematocrit (0.5–0.55) to prevent of post-exchange anaemia and polycythaemia and negative for high titre haemolysins. A number of these evidenced-based best practices; facility for intrauterine blood transfusion, facilities for diagnosis of foetal anaemia in utero, maternal alloantibody testing, elution testing, facility for gamma irradiation of blood, CMV testing of donors units, testing of donor units for other clinical significant red cell antigens apart from ABO and Rhesus D, SAGM units and leucodepletion of donor units are often not available in Nigeria and most developing countries. This failure in stewardship by government in these countries limits the delivery of best possible care in the management of HDFN and appropriate transfusion in these patients.

4.4 Absence of facility to monitor the antibody titre and quantification in pregnant women who have developed HDFN causing alloantibody during pregnancy

Haemolytic Disease of the Foetus and Newborn (HDFN) is the destruction of foetal and newborn red cells by maternally derived alloantibody directed against a red cell antigen often inherited from the father and expressed on foetal cells. These maternal IgG antibodies are low molecular weight and can pass through the placenta barrier into the foetal circulation and bind to the corresponding foetal red cell antigen. The resultant maternal IgG-coated cells interact with foetal macrophages in resulting in their removal by the foetal spleen. This often results in anaemia, erthroblastosis with compensatory erythropoiesis resuming in the foetal liver and spleen, resulting in hepatosplenomegaly and portal hypertension. Maternal antibody screening is undertaken to detect clinically significant antibodies, which might affect the foetus and/or newborn, and to detect antibodies that may cause problems with the provision of compatible blood products for the woman and for the foetus/newborn. Approximately 1% of pregnant women are found to have clinically significant red cell antibodies [107–109]. The detection of clinically significant antibodies among antenatal women plays an important role in transfusion safety and preventing HDFN. A previous study among antenatal multiparous women in Jammu region of India indicated that the commonly observed alloantibodies were anti-D, anti-E, anti-C and anti-K [110]. Once an antibody has been detected, it is identified by carrying out a panel test. If the antibody specificity is one that has the potential to cause HDFN, the titre/quantification must be carried out periodically. Antibody quantification and titration is performed on patients' samples, to support the prediction and management of HDFN and ABO mismatched organ transplant. Antibody quantification of anti-D, and anti-c for management of HDFN. Antibody titration in ABO mismatched transplant is carried out to allow clinical assessment of the possibility of carrying out an ABO mismatched transplant, and monitoring of treatment to reduce antibody titre in preparation for ABO mismatched transplant. All clinically significant maternal antibodies detected during antenatal booking or follow up visit must be referred to the reference laboratory for confirmation of specificity, determination of the possibility of the antibody to cause HDFN and for and titre/quantification to allow for the monitoring of antibody. Evidence based best practice in developed countries requires the monitoring of women with red cell antibodies of specificities known to have potential to cause significant HDFN (anti-D, anti-c and anti-K), covering frequency of testing (every 4 weeks from booking until 28 weeks and every 2 weeks there after till delivery), measurement of antibody concentration (by quantification or titration as appropriate), referral to a foetal medicine specialist and follow-up required post-delivery. Antibodies that require quantification include anti-c, anti D, anti c + D, and anti-c + E. The antibodies that require titration include Anti-e, c, E, C+ e, G, CW, Fya, Fyb, K, k, Kpa, Kpb, Jsa, Jsb, M, S, and s. Antibody titration (ABT) is a semi quantitative method used to detect the strength of an alloantibody/antibodies present in the patient's plasma [111]. Antibody titration is used prenatally to screen for risk of HDFN, haemolytic transfusion reactions and assessment in solid organ or haematopoietic stem cell transplant. To assess risk for HDFN, the titre of the clinically significant alloantibody is performed. When the antibody and the titre strength are identified, ABT is periodically performed throughout pregnancy, whereby plasma samples are compared in parallel with the previously frozen samples to determine if the titre or strength of the antibody is static, decreasing or increasing. Obstetricians can use this information in conjunction with middle cerebral artery Doppler assessment to determine the extent to

which the developing infant is affected, the presence of foetal anaemia, decision to carry out intrauterine transfusion as well as decision on whether to deliver the baby earlier to allow for a better and less risky physical management rather than ultrasound-guided in utero management [112]. Colour Doppler ultrasonography a non-invasive technique is currently being used to assess cerebral artery blood flow [113]. Increased cerebral artery blood flow is an indicator of foetal anaemia [114]. Invasive procedures such as amniocentesis or cordocentesis, with subsequent analysis of amniotic fluid and foetal blood sampling obtained by ultrasonography guided procedure are invasive and significantly risky with potential attendant negative effect to the foetus. Accepted titration methodologies include the tube and gel column technology method [115]. A difference of 2 dilutions or a score of 10 is usually an indication of significant change in antibody production. The antibody titre/quantification often rises as a result of re-exposure of the maternal immune system to red cell antigen following the initial sensitising events that occur during pregnancy. Every re-exposure to the initial offending antigen from the foetus to the mother's immune system often becomes booster doses. These booster doses often result in more antibody production, attendant increase in the titre and quantification. Antibody titration is also commonly used for screening blood products, particularly platelets and plasma as well as group O donor blood intended for transfusion to blood group A, B and AB patients. This is to decrease the risk of haemolytic transfusion reactions due to passive anti-A/anti-B antibodies. The titre of group O products is determined and those with high titers [51, 114] are labelled and used for group O individuals only while those negative for HT haemolysin can be given to A, B and AB individuals [116]. Also, ABT also has a potential role in preventing graft rejection in ABO-incompatible solid organ transplants (heart, liver, and lung) [117] as well as in erythroid engraftment after haematopoietic progenitor cell transplants [118]. Previous report indicates that the performance of antenatal titrations by column agglutination technology (CAT) is at least as sensitive as the performance by the indirect antiglobulin test (IAT) tube method. CAT was found to show greater sensitivity than IAT tube when dealing with anti-c compared to titrating Anti-K. The CAT method also appears to be more sensitive for detecting samples that require referral to the obstetrician [119]. Evidenced-based best practice requires that all pregnant women are tested for the presence of alloantibodies and its titers/quantification during pregnancy as a way of calculating the risk of developing HDFN [120]. Guidelines of the British Committee for standards in Haematology requires that, all pregnant women should be ABO and D antigen typed and screened for presence of alloantibodies at booking and at the 28th week of gestation [121]. Similarly, in the Netherlands, it has been mandatory to screen all pregnant females for presence of alloantibodies [122]. The aim of periodic antibody titration and quantification (every 4 weeks from detection at booking till 28 weeks and every 2 weeks thereafter until delivery) is to determine whether the titre/quantification is static, decreasing or increasing. A static titre/quantification shows that there is no increase in severity over time, a dropping titre/quantification indicate a low risk for HDFN while a rising titre/quantification is an indication of a potentially severe HDFN. For anti-D quantification a level of 4–15 IU indicates a moderate risk while a level > 15 IU indicates a high risk for HDFN. For anti-c a level > 7.5 IU indicates a high risk of HDFN. For anti-K, a titre of 1:32 or greater is indicative of HDFN risk. Generally, a 4-fold increase in the titre of an HDFN-associated alloantibody is an indication of a significant risk for HDFN [68]. In Nigeria and some other African countries alloantibody testing at booking and at 28 weeks gestation is not available, facilities for alloantibody identification and titration/quantification is not available. A number of pregnant women have alloantibodies without knowing and the risk of mortality from HDFN is high.

Nigerian and most of these countries can afford to provide the best possible antenatal care for their pregnant population like it obtains in the developed world. However, lack of political will, failure in stewardship and endemic corruption and misappropriation of the people's resources remains a major challenge to the effective management of the women. African government must rise to the responsibilities and do what is right by implementing evidence-based practice to allow for the effective management of pregnancies associated with clinically significant alloantibodies and its associated HDFN.

4.5 Challenge associated with use of anti-D immunoglobulin for the prevention of haemolytic disease of the foetus and newborn

Alloantibody-D are produced in a woman of child bearing age either as a result of Rhesus D incompatible transfusion (deliberate or erroneous transfusion of D positive donor red cells to Rhesus D negative women) or as a result of prior Rhesus D incompatibility between the mother and the developing foetus. These sensitizations are often as a result of fetomaternal haemorrhage during pregnancy or during delivery. This is often associated with D positive red cells entering into the maternal circulation to sensitise the mother to produce these immune antibody D. This immune D is an IgG class antibody and can potentially cross the placenta in subsequent D positive pregnancies to cause D HDFN. If a Rhesus D negative woman is married to Rh D positive men who are either homozygous or heterozygous for blood group antigen D, there is a 100 and 50% chance respectively of potentially carrying a Rhesus D positive foetus. In such pregnancies the risk of sensitization of the mother during the first pregnancy as a result of fetomaternal haemorrhage (during pregnancy or delivery) of D positive foetal red cell into maternal circulation is high. The recommend that prophylaxis be provided as soon as possible and always within 72 h of the following potentially sensitising event taking place in Rh D negative pregnant women (Amniocentesis, chorionic villus biopsy and cordocentesis, APH, PV bleed during pregnancy, ECV, Abdo trauma (sharp/blunt, open/closed, ectopic pregnancy, evacuation of molar, pregnancy, IUFD, intrauterine intervention during pregnancy (transfusion, surgery, insertion of shunts and laser), miscarriages, TOP, delivery (normal, instrument or C/S) and intraoperative cell salvage) to prevent sensitization and anti-D-related HDFN in subsequent pregnancy. Evidence-based best practice in most developed countries is to prevent this sensitization from taking place by the implementation of the Routine Antenatal Anti-D Prophylaxis (RAADP) programme. This program is alloimmunization prevention where all Rh D negative women are universally offered anti prophylaxis during pregnancy (28 weeks gestation) and following the delivery of a Rhesus positive baby. The implementation of this program has significantly reduced the number of residual alloimmunization from 16% to less than 0.1% [123]. The justification for this implementation is based of scientific evidence that 92% of women who develop an alloantibody-D during pregnancy do so at or after 28 weeks gestation [124, 125].

This implementation has significantly reduced the number of women who are sensitised and by extension the number of D HDFN. However, in countries like Nigeria and some other developing countries where this policy has not been implemented, a significant 14% of unfortunate foetuses are born dead while 50% of those who are born alive suffer from brain injury and neonatal death [126]. This cases of preventable death of Nigerian and African children is a humanitarian emergency that require urgent attention and good will by the Nigerian, African government and people of good will across to globe. Rh_o(D) immune globulin (RhIG) is a medication that can be given to non-sensitised Rhesus D negative pregnant women to prevent Rh D sensitization. The medication is often administered intramuscularly

or intravenously and has a half-life of 12 weeks. Rho(D) immune globulin used for prophylaxis is a human derived fractionated plasma product produced from blood donors who have high levels of anti-D Ig either due to previous sensitization or intentional immunisation of Rh D negative men to produced immune D. This product is significantly virally tested (HBsAg, anti-HIV and HCV RNA) and includes viral inactivation steps in order to further reduce the risk of viral transmission. There is still a small unquantifiable possibility of transmitting prion particularly vCJD or other infectious agents. Product safety data submitted by manufacturers to National Institute of Health and Clinical Excellence technical appraisal guidance 156 (National Institute for Health and Clinical Excellence [127]) indicates a very low rate of adverse event (less than one non-serious per 80,000 doses of anti-D administered) [89]. There is no evidence till date to suggest that prophylactic anti-D administered to Rhesus D negative women during pregnancy is harmful to the foetus. Child birth or delivery is a potential sensitising event that can potentially expose the mother to the Rhesus D positive cells of the baby. Following delivery in these women, the cord blood is tested for ABO, Rh D group and DAT. If the infant is Rhesus D positive, the mother sample taken within 72 h is tested for fetomaternal haemorrhage (flow cytometry of Kleihauer-Betke test). These tests quantify the number of foetal cells that has entered the maternal circulation and thus facilitates the determination of the optimal dosage of prophylactic anti-D to be administered. As a general rule 125iu of anti-D is required to clear 1 ml of foetal cells from maternal circulation. A dose of 300 μg or 1500 IU is often sufficient to prevent alloimmunization after delivery in 99% of cases [128]. If the D-type of a newborn or stillborn is unknown or cannot be determined, a dose of anti-D prophylaxis should be administered. However, if the infant is found Rhesus D negative, prophylaxis will not be required. Evidenced-based best practices in most developed countries as part of their HDFN prevention program recommend the following [92].

In Nigeria and many countries in Africa, these recommendations and best practices are not being implemented. Government in these countries will need to rise up to their responsibilities by implementing these recommendations to prove the sincerity of their resolve to offer their people particularly Rhesus negative women the best possible health care delivery.

4.6 Unaffordability due to high cost of prophylactic anti-D

Anti-D related HDFN often result from the transplacental passage of maternal allo-antibodies directed against foetal red cell antigens inherited from the father affects the foetus or neonate. Majority of the mothers becomes sensitised following small feto-maternal haemorrhages during pregnancy and at delivery of the first Rh D-positive infant. These antibodies can potentially cause HDFN in successive Rh D-positive infants. Implementation of universal access to prophylactic anti-D given during antenatal and post-partum period following the delivery of a Rh D positive baby can help prevent primary Rh D immunisation and risk of HDFN in subsequent pregnancies [129]. It is recommended that routine antenatal anti-D prophylaxis (RAADP) is offered to all non-sensitised pregnant women who are Rh D negative to reduce the risk of sensitization and by extension D-related HDFN [130]. The World Health Organisation (WHO) recommends that antenatal prophylaxis with anti-D immunoglobulin should be given to non-sensitised Rh-negative pregnant women at 28 and 34 weeks of gestation to prevent Rh D alloimmunization. It is estimated that single dose of anti-D can cost around US$ 50 (500 IU) to US$ 87 (1500 IU), depending on the brand and local taxes. Therefore, the cost of antenatal prophylaxis for two 500 IU doses could be as much as US$ 100 per woman. Additional costs will include screening for blood typing in settings where Rh blood tests are not

currently performed [131]. However, providing anti-D prophylaxis universally to all Rh D negative non-sensitised pregnant women is not cheap. The cost benefit analysis of preventing sensitization, HDFN and its related physical disabilities, mental retardation and death of affected children supports investing in the implementation of this policy by responsible government who believes that every life count. Routine antenatal anti-D prophylaxis provides a cost effective intervention for preventing HDFN in non-sensitised Rh D-negative pregnant women [132]. Health economic model indicates a significant cost per quality-adjusted life-year (QALY) gained by the implementation of RAADP given to Rh D-negative primigravidae versus no RAADP is between £9000 and £15,000, and for RAADP given to all RhD-negative women rather than to Rh D-negative primigravidae only is between £20,000 and £35,000. It is suggested that a programme of routine prophylaxis would be cost saving if HDFN were eradicated by its implementation [130]. The National Institute for Health and Clinical Excellence reported that when RAADP for all Rh D-negative women was compared with that for primigravidae, the additional cost per incident of sensitisation prevented ranged from £2900 to £8200 depending on the regimen used. The cost per HDFN-associated foetal loss avoided was £42,000–120,000. It does make economic sense for African government to rise to their responsibilities by proving universally anti-D prophylaxis to all non-sensitised Rhesus D negative women. There is also potential to significantly reduce the cost of implementing RAADP by these governments investing on facilities to non-invasive determination of foetal D genotyping for all non-sensitised Rh D negative pregnant women. This will help identify women who are carrying Rhesus D negative mothers who will not require the prophylaxis. Also, because the prevalence of Rh D negative status is significantly lower among Nigerians (6%) [133] compared to the West ≥15% [134], it is likely to cost African countries a lot less to implement universal access to anti-D prophylaxis [135].

4.7 Challenges associated with the provision of antigen negative red cells to patients with clinically significant red cell antibodies

Evidenced based best practice in management of patients including pregnant women requires that patients are transfused with red cells lacking the group specific antigens to which the recipient alloantibody is specific [136]. This aim of this implementation is to prevent immune mediated destruction of the donor red cells containing the offending antigen [137, 138]. For example, pregnant women who have alloantibody Kell should be given K-negative donor units. The provision of antigen negative blood units for these patients is a special challenge particularly in Nigeria and many other countries in Africa [139]. Although these countries have national blood transfusion services, majority of them are often not fit for purpose. Majority of them test blood donors only for their ABO and Rhesus antigen status [139]. Routine testing of blood donors for other clinically significant red sell antigens are not routinely done. This has a significant implication on haemolytic transfusion reaction and HDFN. In the alloimmuned pregnant women who have low molecular weight immune IgG antibodies, IAT crossmatching should ideally be carried out using donor red cells suspended in low ionic strength saline preferably using highly sensitive column agglutination technique instead of the conventional less sensitive tube methods. Many of these technologies are often not available in most settings in Nigeria and other African countries. Transfusion of antigen positive donor red cells to patient with the group specific antibodies like it potentially happens in Nigeria and some other African countries have significant negative consequences [140]. These antibodies can cause clinically significant haemolytic transfusion reactions, difficulty in cross-matching

blood and getting compatible blood in future, cause decrease in RBC survival and thus negate the aim of red cell transfusion of managing anaemia, improving the quality of life and improving the oxygen carrying capacity of the recipient's blood [141]. Also, there is also the effect of managing donor red cell clearance and the product of red cell breakdown due to haemolysis which often results in multiple organ failure, electrolyte perturbations, coagulopathy and in some severe cases, death [142]. Nigerians and citizens of other African countries deserve the best quality transfusion service like their counterparts in the West. It is the responsibility of African government to work smartly and effectively by avoiding waste and eradicating corruption to ensure that citizens get the best quality health care they deserve.

4.8 Lack of facilities for feto-maternal haemorrhage testing

The accurate detection and quantification of foetal red blood cells (RBCs) in the maternal circulation are necessary for the prevention of Rhesus D alloimmunization among D-negative women because of FMH. It is critical to the administration of adequate amount of anti-D prophylaxis are necessary for the prevention of Rhesus D alloimmunization. As a result of D incompatibility between mother (Rhesus D negative and foetus (D-positive), foetal red cells can enter into maternal circulation and sensitise the mother to produce anti-D alloantibody. These anti-D alloantibodies (small molecular weight IgG antibodies) can pass through the placenta barrier in subsequent D positive pregnancies cause HDFN (haemolysis, foetal anaemia, hydrops foetalis, kernicterus or even death). FMH testing helps obstetrician to determine that a potential sensitising event has taken place and facilitate the administration of adequate amount of prophylactic anti-D (125 IU per 1 ml bleed of foetal red cells into maternal circulation). The widespread use of FMH testing in the evidenced-based provision of adequate immunoprophylaxis with anti-D immunoglobulin has resulted in a significant reduction in the incidence of anti-D related HDFN mortality. Evidence-based best practice implementation of FMH test and provision of immunoprophylaxis with anti-D immunoglobulin in England and Wales has brought about a significant reduction in the number of infants affected with HDFN from affected in 1.2 per 1000 births in 1970 to 0.02 per 1000 births by 1989. There are several methods available for FMH testing (Quantitative and qualitative). Three qualitative methods (micro Du, rosette test, and PEG Du) and two quantitative methods (acid elution and Flow Cytometry) for assessing FMH were evaluated with particular attention given to PEG Du and FC. Of the qualitative techniques, the micro Du test was the least sensitive with 20% false-negative results occurring at 0.5% foetal cells. The PEG Du test was only slightly more sensitive and offered no clinical advantage. The rosette test was the most sensitive, consistently detecting foetal cells at concentrations of 0.25% or greater. Flow Cytometry and acid elution showed similar results, with good correlation obtained between measured and expected quantities of foetal cells ($r = 0.99$ and 0.96, respectively). One of 26 postpartum samples was positive by all screening techniques; acid elution and FC detected 0.3% concentrations of fetal cells and 0.17% [143, 144]. The rosette screen is a highly sensitive in qualitatively detecting 10 mL of foetal whole blood in the maternal circulation. As the screen is reliant on the presence of the D antigen to distinguish foetal from maternal cells, it cannot be used to detect FMH in D-positive mothers or in D-negative mothers carrying a D-negative fetus. The Kleihauer-Betke acid-elution test, the most widely used confirmatory test for quantifying FMH, relies on the principle that foetal RBCs contain mostly foetal haemoglobin (HbF), which is resistant to acid-elution whereas adult haemoglobin is acid-sensitive. Although the Kleihauer-Betke test is inexpensive

and requires no special equipment, it lacks standardisation and precision, and may not be accurate in conditions with elevated F-cells. Flow cytometry is a promising alternative, although its use is limited by equipment and staffing costs. The two well-established confirmatory tests are the Kleihauer-Betke acid-elution assay and flow cytometry. The rosette screen is a highly sensitive FDA approved method to qualitatively detect 10 mL or more of foetal whole blood, or 0.2% foetal cells (volume/volume) in the maternal circulation [143]. The principle is based upon the formation of microscopic foetal D+ aggregates upon incubation of foetal cells with enzyme0-treated group O D-positive indicator RBCs and reagent anti-D serum. The foetal cells must be D-positive and the maternal cells D-negative for the test to be valid. In summary the maternal blood sample is first incubated with anti-D and then washed. The indicator D-positive RBCs are added and the sample is examined under a light microscope. In the presence of anti-D coated foetal D-positive cells, the indicator cells will form aggregates (or rosettes) around the foetal cells. The main limitation of this method is that falsely positive result may occur is the mum has a variant of the D antigen and falsely negative if the foetus or neonate is weak D. Also, mothers that have a positive direct antiglobulin test (DAT) or an autoantibody can produce a false positive result due to the agglutination of the mother's antibody coated red cells.

A positive rosette test is indicative of FMH > 10 mL. In a negative rosette test administration of is 300 μg (1500 IU) of Rh Ig is sufficient to prevent immunisation in 99% of patients. If the rosette test is positive quantification of the FMH by either the Kleihauer-Betke acid elution test or flow cytometry is indicated to determine the optimal dose of prophylactic anti-D immunoglobulin to be administered. The Kleihauer-Betke acid-elution test is an inexpensive method and requires no special equipment and thus can possibly be implemented in developing country like Nigeria and other resource-limited settings. The method is based on the principle that foetal RBCs containing foetal haemoglobin (HbF) to resist acid elution whereas adult haemoglobin does not. When a peripheral smear prepared from a EDTA anticoagulated maternal blood sample is exposed to an acid buffer such as Shepherds stain, haemoglobin in the cytoplasm of maternal red cells are eluted while HbF in the foetal red cells resist acid elution. Subsequent counterstaining with haematoxylin results in red cell containing foetal haemoglobin staining pink while the maternal adult haemoglobin containing red cells appear as ghost. **Figure 1** shows a positive Kleihauer-Betke test. To calculate the number of foetal cells in maternal circulation using Kleihauer-Betke test, get a stained thin film on a microscope and replace one of the objectives of the microscope with a graticule (Miller square) (**Figure 2**). Focus the microscope making sure the graticule is on the top left-hand corner. Select the ×40 lens and count at least 50 fields of the area of the film where the red cells are just touching each other. For each field count all the foetal cells in the large and small square. Count all the maternal cells in the small square. Count fields with at least 25–30 maternal cells. To calculate the total number of maternal cells, add the total number of maternal cells together and multiply by 9. Also add the total number of foetal cells from the 50 fields. The calculation of FMH is done using Mollison's rule.

Mollison's rule = 2400 × number of foetal cells ÷ number of maternal cells

For example, if the number of foetal red cells is 20 and the maternal cells is 2000, the FMH = number of maternal cells = 2000 × 9 = 18,000

25 × 2400/18,000 = 3.3 mls bleed.

Calculation of dose of anti-D. Anti-D administered is calculated by giving 125 IU per ml of FMH. For a 3.3 ml bleed you will have to administer 3.3 × 125 IU = 413 IU of anti-D.

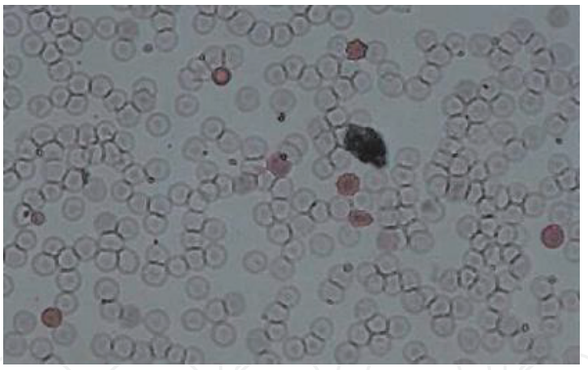

Figure 1.
Positive Kleihauer-Betke test.

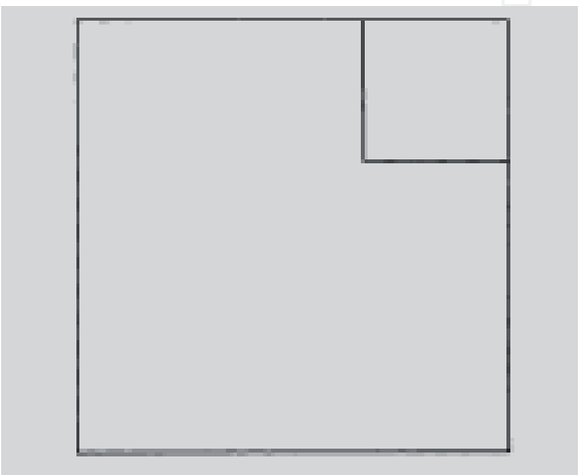

Figure 2.
Diagram of Miller square (graticule).

The Kleihauer-Betke method is a labour intensive and subjective method. The accuracy and precision of this method is hampered by lack of standardisation which can potentially lead to slight variations in result (thickness of blood smear, pipetting skill of analyst, pH variations in the buffer used, inter analyst variations). Over- and underestimation of FMH using the Kleihauer-Betke test has been reported [145, 146]. The disadvantages of this method include; laborious to perform, lacks standardisation, is imprecise and may not be accurate in conditions associated with elevated haemoglobin F containing red cells. However, standardisation of the Kleihauer-Betke test can make the result potentially comparable to result from flow cytometry [147]. Haemoglobin F containing red cells are increased in haemoglobinopathies including sickle cell disease and β-thalassemia and hereditary persistence of foetal haemoglobin (HPFH). In 25% of pregnant women, HbF tend to be increased and can cause false positive results [148, 149]. In pregnant women with conditions such as sickle cell disease, thalassaemia and HPFH an alternative method for FMH testing such as flow cytometry should be employed. Despite its limitations, a good correlation has been reported between the Kleihauer-Betke test and flow cytometry for both small and large FMH [150, 151].

Flow cytometry involves using a flow cytometer using monoclonal antibodies directed against. Flow cytometric determination of FMH is superior to Kleihauer-Betke test for a number of reasons; cytometric methods can accurately distinguish adult foetal haemoglobin containing red cells from foetal RBCs, flow cytometers rapidly analyses a significantly higher number of cells (≥50,000), thus improving

its quantitative accuracy and flow cytometry is automated non-subjective and has greater reproducibility. The only disadvantage is that it requires trained staff to perform test coupled with the fact that it is it is expensive and may not be affordable in low-resource settings.

5. Conclusions

There is paucity of data on the distribution of clinically significant blood group antigens apart from ABO and Rhesus D among Nigerians. Management of Rhesus D negative pregnancies and pregnancies associated with clinically significant alloantibody is suboptimal. This failure in stewardship by the Nigerian government has a significant implication for Haemolytic Disease of the Foetus and Newborn and haemolytic transfusion reaction. There is need to introduce routine screening of pregnant women in Nigeria for clinically significant red cell antibodies to facilitate the effective management of HDFN as well as prevent HTR by enabling the selection of antigen negative red cells for women who have alloantibodies and require a red cell transfusion.

5.1 Recommendations

We recommend that the Nigerian government should implement the following evidence-based best practices (British Committee for Standards in Haematology [152–155].

1. That the Nigerian government should fulfil her obligations under goal 3.8 of the Universal Health Coverage initiative and the Sustainable Development goal 3.b which emphasises the need for access to safe, effective, quality and affordable essential medicines for all by implementing universal access to anti-D prophylaxis for previously unsensitized Rhesus D negative pregnant women.

2. Screen donor units for clinically significant antigen status to facilitate the selection of antigen negative donor units for transfusion to patients with a clinically significant alloantibody.

3. Routinely screening all blood group O donors whose units are intended for transfusion to non-O recipients for high titre haemolysin.

4. Anti-D immunoglobulin should be available in cases of potentially sensitising events such as amniocentesis, cordocentesis, antepartum haemorrhage, vaginal bleeding during pregnancy, external cephalic version, abdominal trauma, intrauterine death and stillbirth, in utero therapeutic interventions, miscarriage, and therapeutic termination of pregnancy.

5. All Rhesus D negative women having a termination of pregnancy (abortion) must be tested for Rhesus D group and those found to be Rhesus D negative must be offered prophylaxis following the procedure.

6. Routine foetal genotyping using non-invasive testing of maternal sample to identify Rhesus negative pregnant women who are carrying Rhesus negative infants for whom prophylaxis is not indicated.

7. Facilities for intrauterine transfusion of infants who become anaemic in utero as a result of HDFN and require a red cell transfusion. Facilities to test donor

red cells to ensure that they meet the requirements for intrauterine, exchange and top up transfusion.

8. Facilities for the implementation of alloantibody titration and quantification should be provided for pregnant women with a clinically significant, HDFN causing alloantibody.

9. There is need for the provision of facilities for fetomaternal haemorrhage testing to facilitate the administration of optimal amount of anti-D prophylaxis following a sensitising event to prevent HDFN.

10. Knowledge of anti-D prophylaxis and management of women with clinically significant antibodies among Obstetricians, Laboratory of Biomedical Scientist, Midwives, Traditional Birth Attendants, Pharmacists and Nurses in Nigeria and other countries in Africa needs to be improved. This will facilitate quality antenatal and postnatal care offered particularly to Rh-negative pregnant women and those with clinically significant alloantibodies.

11. That anti-D prophylaxis be provided in Rhesus D negative pregnant pregnancies <12 weeks gestation, following ectopic pregnancy, molar pregnancy, therapeutic termination of pregnancy. FMH haemorrhage (FMH) testing is often not required for sensitising events at <12 weeks gestation.

12. That for all potentially sensitising events occurring between 12- and 20-weeks' gestation, prophylaxis should be administered within 72 h of the event without need for FMH testing.

13. That for potentially sensitising events after 20 weeks gestation, prophylaxis should be administered within 72 h of the event along with testing for FMH. This is to determine if additional dose of anti-D Ig is required for a significant bleed.

14. That in the event of an intrauterine foetal death (IUFD), where no sample can be obtained from the baby or foetus, an appropriate dose of prophylactic anti-D should be administered to Rhesus D negative, previously non-sensitised women within 72 h of the *diagnosis of IUFD*, irrespective of the time of subsequent delivery.

15. That following intra-operative cell salvage (ICS) during Caesarean section in D negative, previously non-sensitised women, and where cord blood group is confirmed as D positive (or unknown), a minimum dose of 1500 IU prophylactic anti-D should be administered following the re-infusion of salvaged red cells. Also, the maternal sample should be taken for estimation of FMH 30–45 min after reinfusion to confirm if a further dose of anti-D Ig is required.

16. That in cases of therapeutic termination of pregnancy (abortion), whether by surgical or medical methods, and regardless of gestational age, all previously non-sensitised D negative women should receive a dose of prophylactic anti-D within 72 h of having the procedures.

17. That anti-D prophylaxis should be administered to all cases of ectopic pregnancy in previously non-sensitised, D negative women regardless of the mode of management.

18. That there is significant potential for sensitisation in cases of molar pregnancy. Anti-D prophylaxis should be administered to all cases of molar pregnancy in previously non-sensitised, D negative women.

19. That D negative women presenting with continual uterine bleeding between 12- and 20-weeks' gestation should be offered anti-D prophylaxis at a minimum of 6 weekly intervals.

20. That under normal circumstances, D negative platelets should be transfused to D negative females or women of child bearing age, who need a platelet transfusion. Occasionally, if the appropriate product is not available or its availability is likely to cause unacceptable delay and harm, it may be necessary to transfuse D positive platelets. In these situations, prophylaxis against possible sensitisation by the D positive red cells contaminating the platelet product should be given. A dose of 250 IU anti-D immunoglobulin is sufficient to cover up to five adult therapeutic doses of D positive platelets given over a 6-weeks period.

Author details

Erhabor Osaro*, Erhabor Tosan, Adias Teddy Charles
and Iwueke Ikechukwu Polycarp
Department of Haematology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria

*Address all correspondence to: n_osaro@yahoo.com

References

[1] Bakare AS, Olubokun S. Health care expenditure and economic growth in Nigeria: An empirical study. Journal of Emerging Trends in Economics and Management Sciences. 2011;**2**(2):83-87

[2] Uneke C, Ogbonna A, Ezeoha A, Oyibo P, Onwe F, Ngwu B, et al. The Nigeria health sector and human resource challenges. International Journal of Health. 2007;**8**(1):1-5

[3] Duru EJ, Nwagbos CI. The problems and prospects of public health care development in Nigeria's local government system. Global Journal of Social Sciences. 2007;**6**(1):51-56

[4] United Nations. Moving Together to Build a Healthier World Key Asks from the UHC Movement UN High-Level Meeting on Universal Health Coverage. UHC2030. Available from: https://www.uhc2030.org/fileadmin/uploads/uhc2030/Documents/UN_HLM/UHC_Key_Asks_final.pdf [Accessed: October 10, 2019]

[5] Wagner AK, Graves AJ, Reiss SK, Lecates R, Zhang F, Ross-Degnan D. Access to care and medicines, burden of health care expenditures, and risk protection: Results from the World Health Survey. Health Policy. 2011;**100**:151-158

[6] WHO. The World Health Report—Health Systems Financing: The Path to Universal Coverage. Geneva: World Health Organization; 2010

[7] ISBT. Table of Blood Group Systems. International Society of Blood Transfusion. 2014. Retrieved 19 February 2016

[8] Marcela C, Antole L. Antigens in human blood. In: Postgraduate Haematology. 4th ed. Boston, Mass, USA: Butterworth-Heinemann; 1999. pp. 186-189 (chapter 10)

[9] Giangrande PL. The history of blood transfusion. British Journal of Haematology. 2000;**110**(4):758-767

[10] Landsteiner K. On agglutination of normal human blood. Transfusion. 1901;**1**(1):5-8

[11] Anifowoshe AT, Oyeyemi BF, Iyiola OA, Ahmed IO, Akinseye KM. Gene frequencies of ABO and Rh (D) blood group alleles in Minna, North-Central, Nigeria. Nigerian Journal of Pure and Applied Sciences. 2015;**28**:2644-2657

[12] Bakare AA, Azeez MA, Agbolade JO. Gene frequencies of ABO and rhesus blood groups and haemo globin variants in Ogbomosho, South-West Nigeria. African Journal of Biotechnology. 2006;**5**:224-229

[13] Enosolease ME. Distribution of ABO and Rh_D blood groups in the Benin area of implication for regional blood transfusion. Asian Journal of Transfusion Science. 2008;**2**(1):3-5

[14] Falusi AG, Ademowo OG, Latunji AC, Okeke C, Olatunji PO, Onyekwere TO, et al. Distribution of ABO and Rh genes in Nigeria. African Journal of Medicine and Medical Sciences. 2000;**29**:23-26

[15] Hassan A, Babadoko AA, Ahmed AJ, Isa HA, Suleiman AM. The pattern of distribution of ABO blood groups in North Western Nigeria. Annals of Nigerian Medicine. 2005;**1**(2):17-18

[16] Iyiola OA, Igunnugbemi OO, Bello OG. Gene frequencies of ABO and Rh(D) blood group alleles in Lagos, South-West Nigeria. Egyptian Journal of Medical Human Genetics. 2012;**13**:147-153

[17] Iyiola OA, Igunnugbemi OO, Raheem UA, Anifowoshe AT. Gene

frequencies of ABO and Rh(D) blood group alleles in Ilorin, North-Central Nigeria. World Journal of Biological Research. 2011;**4**(1):6-14

[18] Omotade OO, Adeyemo AA, Kayode CM, Falade SL, Ikpeme S. Gene frequencies of ABO and Rh (D) blood group alleles in a healthy infant population in Ibadan, Nigeria. West African Journal of Medicine. 1991;**18**(4):294-297

[19] Anifowoshe AT, Owolodun OA, Akinseye KM, Iyiola OA, Oyeyemi BF. Gene frequencies of ABO and Rh blood groups in Nigeria: A review. Egyptian Journal of Medical Human Genetics. 2017;**18**(3):205-210

[20] Benahadi A, Alami R, Boulahdid S, Adouani B, Laouina A, Mokhtari A, et al. Distribution of ABO and Rhesus D blood antigens in Morocco. Internet Journal of Biological Anthropology. 2013;**6**(1):1-6

[21] Garratty G, Glynn SA, McEntire R. ABO and Rh (D) phenotype frequencies of different racial/ethnic groups in the United States. Transfusion. 2004;**44**(5):703-706

[22] Hamed CT, Bollahi MA, Abdelhamid I, Mahmoud M, Ba B, Ghaber S, et al. Frequencies and ethnic distribution of ABO and Rh (D) blood groups in Mauritania: Results of first nationwide study. International Journal of Immunogenetics. 2012;**39**(2):151-154

[23] Ndoula ST, Noubiap JJN, Nansseu JRN, Wonkam A. Phenotypic and allelic distribution of the ABO and Rhesus (D) blood groups in the Cameroonian population. International Journal of Immunogenetics. 2014;**41**(3):206-210

[24] Said N, BenAhmed F, Doghri A, Ghazouani E, Layouni S, Gritli V, et al. The ABO system polymorphism in Tunisian blood. Transfusion Clinique et Biologique. 2003;**10**:331

[25] Tesfaye K, Petros Y, Andargie M. Frequency distribution of ABO and Rh (D) blood group alleles in Silte Zone, Ethiopia. Egyptian Journal of Medical Human Genetics. 2015;**16**(1):71-76

[26] Torabizade Maatoghi J, Paridar M, Mahmodian Shoushtari M, Kiani B, Nori B, Shahjahani M, et al. Distribution of ABO blood groups and rhesus factor in a large-scale study of different cities and ethnicities in Khuzestan province, Iran. Egyptian Journal of Medical Human Genetics. 2016;**17**(1):105-109

[27] Loua A, Lamah MR, Haba NY, Camara M. Frequency of blood groups ABO and Rhesus D in the Guinean population. Transfusion Clinique et Biologique. 2007;**14**:435

[28] Randriamanantany ZA, Rajaonatahina DH, Razafimanantsoa FE, Rasamindrakotroka MT, Andriamahenina R, Rasoarilalamanarivo FB, et al. Phenotypic and allelic profile of ABO and Rhésus D blood group system among blood donor in Antananarivo. International Journal of Immunogenetics. 2012;**39**(6):477-479

[29] Chandra T, Gupta A. Frequency of ABO and Rhesus blood groups in blood donors. Asian Journal of Transfusion Science. 2012;**6**(1):52-53

[30] Dewan G. Comparative frequency and allelic distribution of ABO and Rh (D) blood groups of major tribal communities of southern Bangladesh with general population and their determinants. Egyptian Journal of Medical Human Genetics. 2015;**16**(2):141-147

[31] Causil-Vargas L, Gómez-Mercado L, Otero-Díaz D, Moscote-Blanco L, Monterrosa-Mestra L, Burgos-Flórez D, et al. Frecuencia de grupos sanguíneos

(sistema ABO) entre estudiantes de la universidad de córdoba sede berástegui. Ciencia e Investigación Medico Estudiantil Latinoamericana. 2016;**21**(1):24-26

[32] Kayiran SM, Oktem O, Kayiran PG, Paloglu E, Gurakan B. Frequency of ABO and Rhesus blood groups among neonates born at a private hospital in Istanbul. The Southeast Asian Journal of Tropical Medicine and Public Health. 2012;**43**(2):467-470

[33] Olaniyan TO, Ajibola BM, Rasong H, Dare BJ, Shafe MO. Blood group and rhesus factor pattern among indigenes of FCT, Abuja, Nigeria. Journal of Community Medicine and Health Education. 2013;**3**(3):208

[34] Adeyemo OA, Soboyejo OB. Frequency distribution of ABO, RH blood groups and blood genotypes among the cell biology and genetics students of University of Lagos, Nigeria. African Journal of Biotechnology. 2006;**5**(22):2062-2065

[35] Alimba CG, Adekoya KO, Oboh BO. Prevalence and gene frequencies of phenylthiocarbamide (PTC) taste sensitivity, ABO and Rhesus factor (Rh) blood groups, and haemoglobin variants among a Nigerian Population. Egyptian Journal of Medical Human Genetics. 2010;**11**(2):153-158

[36] Oluwadare I, Shonekan S. ABO and Rhesus 'D' blood type distribution in students admitted into MoshoodAbiola Polytechnic, Abeokuta, Nigeria in 2006. African Journal of Biotechnology. 2008;**7**(11):1641-1643

[37] Temitayo OI, Timothy SO. Frequency distribution of haemoglobin variants and rhesus blood groups among pregnant. American Medical Journal. 2013;**4**(1):78-81

[38] Olubayode B, Dennis SA, Abiola OA. Distribution of ABO and rhesus blood groups among medical students in Bowen University, Iwo, Nigeria. Annals of Biological Research. 2013;**4**(11):1-6

[39] Akhigbe RE, Ige SF, Adegunlola GJ, Adewumi MO, Azeez MO. Malaria, haemoglobin genotypes abd ABO blood group genotypes in Ogbomoso, Nigeria. International Journal of Tropical Medicine. 2011;**6**(4):73-76

[40] Famoni PO, Adekoya-Benson T, Ajala O, Agbaje A, Olatimehin O, Adeosun OC, et al. Frequencies of ABO blood groups among blood donors in Ado-Ekiti and its metropolis, South-West, Nigeria. International Journal of Healthcare Sciences. 2015;**2**(2):126-129

[41] Akpan EB, Iwah-Udo IS. Studies of the distribution of ABO blood groups and Rhesus factors in Uyo, Akwa Ibom State, Nigeria. African Journal of Applied Zoology and Environmental Biology. 2006;**8**:159-161

[42] Egesie UG, Egesie OJ, Usar I, Johnbull TO. Distribution of ABO, Rhesus blood groups and haemoglobin electrophoresis among the undergraduate students of Niger Delta University Nigeria. Nigerian Journal of Physiological Sciences. 2008;**23**:5-8

[43] Odokuma EI, Okolo AC, Aloamaka PC. Distribution of Abo and Rhesus blood groups in Abraka, Delta State, Nigeria. Nigerian Journal of Physiological Sciences. 2007;**22**(1-2):89-91

[44] Erhabor O, Adias TC, Jeremiah ZA, Hart ML. Abnormal hemoglobin variants, ABO, and Rhesus blood group distribution among students in the Niger Delta of Nigeria. Pathology and Laboratory Medicine International. 2010;**2**:41-46

[45] Akinnuga AM, Bamidele O, Amosu AM, Ugwah GU. Distribution of ABO and Rh blood groups among major

ethnic groups of medical students of Madonna University Teaching Hospital, Elele, Nigeria. Asian Journal of Medical Sciences. 2011;**3**(3):106-109

[46] Jeremiah ZA. Abnormal haemoglobin variants, ABO and Rh blood groups among students of African descent in Port-Harcourt, Nigeria. African Health Sciences. 2006;**6**(3):177-181

[47] Chima OK, Mohammed TB, Aisha K, Alhaji SA, Muhammad BM, Kwaru AH. ABO and rhesus blood groups among blood donors in Kano, North-Western Nigeria. Nigerian Journal of Basic and Clinical Sciences. 2012;**9**(1):11-13

[48] Erhabor O, Isaac IZ, Saidu A, Ahmed HM, Abdulrahaman Y, et al. The distribution of ABO and rhesus blood groups among residents of Gusau, Zamfara State, North Western Nigeria. Research and Reviews: Journal of Medical and Health Sciences. 2013;**2**(4):58-63

[49] Obeagu EI, Ogbodo OR, Onyenweaku F, Emelike CU, Udochukwu AI. Frequency distribution of ABO, Rh blood groups and blood genotypes among the students and staff of Michael Okpara University of Agriculture, Umudike Abia State, Nigeria. International Journal of Research and Reviews in Pharmacy and Applied Science. 2013;**3**(4):561-565

[50] Ugwu ZI. Pattern of ABO and Rhesus blood group distribution among students of Ebonyi State University, Abakaliki, South Eastern Nigeria. Asian Journal of Medical Sciences. 2016;7(1):101-104

[51] Abdulazeez AA, Alo EB, Rebecca SN. Carriage rate of human immunodeficiency virus (HIV) infection among different ABO and rhesus blood groups in Adamawa state, Nigeria. Biomedical Research. 2008;**19**(1):41-44

[52] 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 Medical Journal. 2014;**11**(2):86-91

[53] Cerny T, Fey M, Oppliger R, Castiglione M, Nachbur B, Gertsch M, et al. Prevalence of the rhesus-negative phenotype in Caucasian patients with small-cell lung cancer (SCLC). International Journal of Cancer. 1992;**52**:504-506

[54] Lin M, Wang CL, Chen FS, Ho LH. Fatal haemolytic transfusion reaction due to anti-Ku in a K null patient. Immunohematology. 2003;**19**(1):19-21

[55] Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA. Inhibition of erythroid progenitor cells by anti-Kell antibodies in foetal alloimmune anaemia. New England Journal of Medicine. 1998;**338**(12):798-803

[56] Vaughan JI, Warwick R, Letsky E, Nicolini U, Rodeck CH, Fisk NM. Erythropoietic suppression in fetal anemia because of Kell alloimmunization. American Journal of Obstetrics and Gynecology. 1994;**171**(1):247-252

[57] Erhabor O, Ladan MA, Zama I, Ahmed Y, Mairo H. Distribution of Kell phenotype among pregnant women in Sokoto, North Western Nigeria. The Pan African Medical Journal. 2015;**21**:301

[58] Ugboma HA, Nwauche CA. Kell blood group antigen in Port Harcourt, Nigeria—A pilot study. Port Harcourt Medical Journal. 2009;**4**(2). ISSN 0795-3038

[59] Adewoyin AS, Lee GM, Adeyemo TA, Awodu OA. Rh and

Kell blood group antigen prevalence in a multi-ethnic cohort in Nigeria: Implications for local transfusion service. Immunohematology. 2018;**34**(2):61-65

[60] Lamba DS, Kaur R, Basu S. Clinically significant minor blood group antigens amongst north Indian donor population. Advances in Hematology. 2013;**2013**:215454

[61] Redman CM, Russo D, Lee S. Kell, Kx and the McLeod syndrome. Baillière's Clinical Haematology. 1999;**12**(4):621-635

[62] Yusuf Jamoh B, Gwaram BA, Abdullahi S, Tasiu M. 12th World Hematologists Congress; March 15-16, 2018; London, UK. 2018

[63] Erhabor O, Shehu CE, Alhaji YB, Yakubu A. Duffy red cell phenotypes among pregnant women in Sokoto, North Western Nigeria. Journal of Blood Disorders & Transfusion. 2014;**5**:223

[64] Okwesili A, Sani AB, Onuigwe F, Hauwa B, Bagudo A, Ibrahim K, et al. Prevalence of rhesus C and E phenotype among pregnant women attending antenatal clinic in Usmanu Danfodiyo University Teaching Hospital, Sokoto. Journal of Gynecology and Neonatal Biology. 2016;**2**(1):13-16

[65] Jeremiah ZA, Odumody C. Rh antigens and phenotype frequencies of the Ibibio, Efik, and Ibo ethnic nationalities in Calabar, Nigeria. Immunohematology. 2005;**21**(1):21-24

[66] Jeremiah ZA, Biribo AA, Adias TC, Uko EK. Uncommon Rh phenotypes in a cross section of Nigerian antenatal women: Implications for molecular genotyping of blood groups. Journal of Blood Disorders & Transfusion. 2012;**S10**:001

[67] Siransy BL, Dembele B, Sekongo Y, Abisse S, Konaté S, Sombo M. Phenotypic Profile of Rh and Kell Blood Group Systems among Blood Donors in Cote d'Ivoire, West Africa. Journal of Blood Transfusion. 2014;**2014**:4

[68] Erhabor O, Adias TC. From whole blood to component therapy: The economic, supply/demand need for implementation of component therapy in sub-Saharan Africa. Transfusion Clinique et Biologique. 2011;**18**(5-6):516-526

[69] Ahmed OA, Agomo PU, Olukoya DK, Esan GJ. The prevalence of ABO blood group antigens and antibodies in Lagos State, Nigeria. African Journal of Medicine and Medical Sciences. 1993;**22**:49-53

[70] Emeribe AO. The status of alpha and beta haemolysins in Nigerian blood donors. East African Medical Journal. 1990;**67**(3):205-208

[71] Obisesan OA, Ogundeko TO, Iheanacho CU, Abdulrazak T, Idyu VC, Idyu II, et al. Evaluation of alpha (α) and beta (β) haemolysin antibodies incidence among blood group 'O' donors in ATBUTH Bauchi-Nigeria. American Journal of Clinical Medicine Research. 2015;**3**(3):42-44

[72] Okafor LA, Enebe S. Anti-A and anti-B haemolysins, dangerous universal blood donors and the risk of ABO antagonism in a Nigerian community. Tropical and Geographical Medicine. 1985;**37**:270-272

[73] Olawumi HO, Olatunji PO. Prevalence and titre of alpha and beta haemolysins in blood group 'O' donors in Ilorin. African Journal of Medicine and Medical Sciences. 2001;**30**(4):319-321

[74] Oyedeji OA, Adeyemo TA, Ogbenna AA, Akanmu AS. Prevalence of anti-A and anti-B hemolysis among blood group O donors in Lagos.

Nigerian Journal of Clinical Practice. 2015;**18**:328-332

[75] Uko EK, Erhabor O, Ahmed HM, Isaac IZ, Abdulrahaman Y, Wase A, et al. Prevalence of high titre alpha and beta haemolysins among blood donors in Sokoto, North Western Nigeria. International Journal of Medical Sciences and Health Care. 2013;**1**(11):1-7

[76] Castilho L. ABO isohemagglutinin titration or hemolysin test: What should we do to reduce the risk of passive hemolysis? Revista Brasileira de Hematologia e Hemoterapia. 2015;**37**(4):215-216

[77] Mathai J, Sindhu PN, Sulochana PV, Sathyabhama S. Haemolysin test for characterization of immune ABO antibodies. The Indian Journal of Medical Research. 2003;**118**:125-128

[78] Nadarajan SN, Hlaing AA, Maung TH, Jeyajoti I, Kyu TN, Ranjana KJ, et al. Incidence of red cell alloantibodies in a multi-ethnic hospital patient population. Vox Sanguinis. 2007;**93**:63-64

[79] Schonewille H, van de Watering LMG, Loomans DSE, Brand A. Red blood cell alloantibodies after transfusion: Factors influencing incidence and specificity. Transfusion. 2006;**46**:250-256

[80] Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion dependent thalassemia patients of predominantly Asian descent. Blood. 2000;**96**:3369-3373

[81] Jan RH, Yu LC, Wen SH, Tsai SS, Lin TY. Incidence of alloantibodies in transfused patients in Eastern Taiwan. Tzu Chi Medical Journal. 2009;**21**:66-69

[82] AmeenR,Al-EyaadiO,Al-ShemmariS, Chowdhury R, Al-Bashir A. Frequency of red blood cell alloantibody in Kuwaiti population. Medical Principles and Practice. 2005;**14**:230-234

[83] Makarovsk-Bojadzieva T. Blood group antibodies that cause problems in pretransfusion testing. Blood Banking and Transfusion Medicine. 2004;**2**:89-93

[84] Al-Joudi F, Ali AB, Ramli MB, Ahmed S, Ismail M. Prevalence and specificities of red cell alloantibodies among blood recipients in the Malaysian state of Kelantan. Asian Journal of Transfusion Science. 2011;**5**(1):42-45

[85] Yousuf R, Abdul Aziz S, Yusof N, Leong CF. 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

[86] Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. Transfusion Medicine Reviews. 2007;**21**:58-71

[87] Illanes S, Soothill P. Management of red cell alloimmunisation in pregnancy: The non-invasive monitoring of the disease. Prenatal Diagnosis. 2010;**30**(7):668-673

[88] Tong KF, Tse KT, Lee AW, Mak CS, So CC. Is antenatal antibody screening worthwhile in Chinese. British Journal of Haematology. 1997;**97**:917-919

[89] 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

[90] Urbaniak SJ, Greiss MA. Rh D haemolytic disease of the fetus and the newborn. Blood Reviews. 2000;**14**:44-61

[91] MacKenzie IZ, Bowell P, Gregory H, Pratt G, Guest C, Entwistle CC. Routine antenatal rhesus D immunoglobulin prophylaxis: The results of a prospective 10-year study. British Journal of Obstetrics and Gynaecology. 1999;**106**:492-497

[92] Qureshi H, Massey E, Kirwan D, Davies T, Robson S, White J, et al. BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the foetus and newborn. Transfusion Medicine. 2014;**24**(1):8-20

[93] Koelewijn JM, de Haas M, Vrijkotte TGM, van der Schoot CE, Bonsel GJ. Risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis. BJOG. 2009;**116**:1307-1314

[94] Koelewijn JM, 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**(5):655-664

[95] Moise KJJ. Management of rhesus alloimmunization in pregnancy. Obstetrics and Gynecology. 2008;**112**:164-176

[96] Reid ME. Application of DNA-based assays in blood group antigen and antibody identification. Transfusion. 2003;**43**:1748-1757

[97] Avent ND. RHD genotyping from maternal plasma: Guidelines and technical challenges. Methods in Molecular Biology. 2008;**444**:185-201

[98] Scheffer PG, van der Schoot CE, Page-Christiaens GC, de Haas M. Noninvasive fetal blood group genotyping of rhesus D, c, E and of K in alloimmunised pregnant women: Evaluation of a 7-year clinical experience. BJOG. 2011;**118**(11):1340-1308

[99] Tóth T, Papp C, Tóth-Pál E, Nagy B, Papp Z. Fetal RhD genotyping by analysis of maternal blood. A case report. Journal of Reproductive Medicine. 1998;**43**(3):219-222

[100] Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, et al. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood. 2000;**95**:12-18

[101] van der Schoot CE, Tax GH, Rijnders RJ, de Haas M, Christiaens GC. Prenatal typing of Rh and Kell blood group system antigens: The edge of a watershed. Transfusion Medicine Reviews. 2003;**17**(1):31-44

[102] Chan FY, Cowley NM, Wolter L, Stone M, Carmody F, Saul A, et al. Prenatal RHD gene determination and dosage analysis by PCR: Clinical evaluation. Prenatal Diagnosis. 2001;**21**(4):321-326

[103] Hromadnikova I, Vesela K, Benesova B, Nekovarova K, Duskova D, Vlk R, et al. Non-invasive fetal RHD and RHCE genotyping from maternal plasma in alloimmunized pregnancies. Prenatal Diagnosis. 2005;**25**(12):1079-1083

[104] Clausen FB, Christiansen M, Steffensen R, et al. Report of the first nationally implemented clinical routine screening for fetal RHD in D– pregnant women to ascertain the requirement for antenatal RhD prophylaxis. Transfusion. 2012;**52**:752-758

[105] Lindemburg ITM, van Kamp IL, van Zwet EW, et al. Increased perinatal loss after intrauterine transfusion for alloimmune anaemia before 20 weeks of gestation. BJOG: An International Journal of Obstetrics and Gynaecology. 2013;**120**:847-852

[106] Rodeck CH, Deans A. Red cell alloimmunisation. In: Rodeck CH, Whittle MJ, editors. Fetal Medicine: Basic Science and Clinical Practice.

2nd ed. London: Churchill Livingstone; 2008. pp. P559-P577

[107] Howard H, Martlew V, McFaden I, Clarke C, Duguid J, Bromilow I, et al. Consequences for fetus and neonate of maternal red cell Allo-immunisation. Archives of Disease in Childhood—Fetal and Neonatal Edition. 1998;**78**:F62-F66

[108] 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**(5):941-952

[109] Smith HM, Shirey RS, Thoman SK, Jackson JB. Prevalence of clinically significant red blood cell alloantibodies in pregnant women at a large tertiary-care facility. Immunohematology. 2013;**29**:127-130

[110] Meena S, Renu B, Naveen A, Vijay S. Prevalence, specificity and titration of red cell alloantibodies in multiparous antenatal females at a tertiary care Centre from North India. Indian Journal of Hematology and Blood Transfusion. 2016;**32**(3):307-311

[111] Finck R, Lui-Deguzman C, Teng SM, Davis R, Yuan S. Comparison of a gel microcolumn assay with the conventional tube test for red blood cell alloantibody titration. Transfusion. 2013;**53**(4):811-815

[112] Ebbing C, Rasmussen S, Kiserud T. Middle cerebral artery blood flow velocities and pulsatility index and the cerebroplacental pulsatility ratio: Longitudinal reference ranges and terms for serial measurements. Ultrasound in Obstetrics & Gynecology. 2007;**30**(3):287-296

[113] Steiner EA, Judd WJ, Combs M, Watkins K, Weissman P, Mann N, et al. Prenatal antibody titers by the gel test. In: Scientific Abstract, Ortho Clinical Diagnostics. 2002 AABB Annual Meeting Presentation. 2002

[114] Mari G, Deter RL. Non-invasive diagnosis by doppler ultrasonography of foetal anaemia due to maternal red-cell alloimmunization. New England Journal of Medicine. 2000;**342**:9-14

[115] Kumlien G. Comparing the tube and gel techniques for ABO antibody titration, as performed in three European centers. Transplantation. 2007;**84**:S17-S19

[116] Hill EA, Bryant BJ. Comparison of antibody titers in donor specimens and associated AS-1 leukoreduced donor units. Transfusion. 2014;**54**(6):1580-1584

[117] Schwartz J, Winters JL, Padmanabhan A, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis: The sixth special issue. Journal of Clinical Apheresis. 2013;**28**(3):145-284

[118] Booth GS, Gehrie EA, Bolan CD, Savani BN. Clinical guide to ABO incompatible allogeneic stem cell transplantation. Biology of Blood and Marrow Transplantation. 2013;**19**(8):1152-1158

[119] Adriaansen MJ, Perry HE. Validation of column agglutination technology for blood group alloantibody titration. New Zealand Institute of Medical Laboratory Science. 2013;**67**:92-96

[120] Judd WJ, Luban NLC, Ness PM, Silberstein LE, Stroup M, Widmann FK. Prenatal and perinatal immunohematology: Recommendations for serological management of the fetus, newborn, infant and obstetric patients. Transfusion. 1990;**30**(2):175-183

[121] British Committee for Standards in Haematology. Guidelines for blood grouping and red cell antibody testing during pregnancy. 2008

[122] Semmekrot BA, de Man AJ, Boekkooi PF. Irregular blood group antibodies during pregnancy: Screening is mandatory. Nederlands Tijdschrift voor Geneeskunde. 1999;**143**:449-452

[123] Bowman JM. Controversies in Rh prophylaxis. Who needs Rh immune globulin and when should it be given? American Journal of Obstetrics and Gynecology. 1985;**151**(3):289-294

[124] ACOG. Prevention of Rh D Alloimmunization. ACOG Practice Bulletin Number 4. Washington, DC: American College of Obstetricians and Gynaecologists; 1999

[125] Bowman JM. The prevention of Rh immunization. Transfusion Medicine Reviews. 1988;**2**(3):129-150

[126] Provan D, Stasi R, Newland AC, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood. 2010;**115**(2):168-186

[127] National Institute for Health and Clinical Excellence (NICE). Routine antenatal anti-D prophylaxis for women who are rhesus D negative) (National Institute for Health and Clinical Excellence (NICE CG156)). 2008

[128] Klein HG, Anstee DJ. Haemolytic disease of the fetus and newborn. In: Mollison's Blood Transfusion in Clinical Medicine. 11th ed. Oxford: Blackwell; 2005. pp. 496-545

[129] Chilcott J, Lloyd Jones M, Wight J, Forman K, Wray J, Beverley C, et al. A Review of the Clinical Effectiveness and Cost-Effectiveness of Routine Anti-D Prophylaxis for Pregnant Women Who Are Rhesus-Negative. NIHR Health Technology Assessment Programme: Executive Summaries. Southampton (UK): NIHR Journals Library; 2003

[130] National Institute for Health and Clinical Excellence. Overview—Routine antenatal anti-D prophylaxis for Rh D-negative women. 2008

[131] WHO Reproductive Health Library. WHO Recommendation on Antenatal Anti-D Immunoglobulin Prophylaxis. (December 2016). The WHO Reproductive Health Library. Geneva: World Health Organization; 2016

[132] Jim C, Paul T, Myfanwy LJ, Jeremy W, Katie F, Julie W, et al. The economics of routine antenatal anti-D prophylaxis for pregnant women who are rhesus negative. BJOG: An International Journal of Obstetrics and Gynaecology. 2004;**111**(9):903-907

[133] Udomah FP, Isaac IZ, Aliyu N, Erhabor O, Ahmed MH, et al. Haemoglobin electrophoretic patterns, ABO and rhesus D blood groups distribution among antenatal women in Sokoto, Nigeria. Obstetrics and Gynaecology Cases—Reviews. 2015;**2**:034-037

[134] Golassa L, Tsegaye A, Erko B, Mamo H. High Rhesus (Rh (D)) negative frequency and ethnic-group based ABO blood group distribution in Ethiopia. BMC Research Notes. 2017;**10**(1):330

[135] Downe S, Finlayson K, Tunçalp Ö, Gülmezoglu AM. Factors that influence the provision of good quality routine antenatal care services by health staff: A qualitative evidence synthesis. Cochrane Database of Systematic Reviews. 2016;**2016**

[136] Thakral B, Saluja K, Sharma RR, Marwaha N. Red cell alloimmunization in a transfused patient population: A study from a tertiary care hospital in North India. Hematology. 2008;**13**(5):313-318

[137] Bujandrić NB, Grujić JN, Krga-Milanović MM. Transfusion management of patients with red blood cell antibodies. Medicinski Pregled. 2013;**66**(11-12):491-496

[138] Pandey H, Das SS, Chaudhary R. Red cell alloimmunization in transfused patients: A silent epidemic revisited. Asian Journal of Transfusion Science. 2014;**8**(2):75-77

[139] Natukunda B, Schonewille H, van de Watering L, Brand A. Prevalence and specificities of red blood cell alloantibodies in transfused Ugandans with different diseases. Vox Sanguinis. 2010;**98**(2):167-171

[140] Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multi transfused thalassaemic of Indian origin. Haematology. 2010;**15**:174-177

[141] Zimring JC, Welniak L, Semple JW, Ness PM, Slichter SJ, Spitalnik SL, et al. Current problems and future directions of transfusion-induced alloimmunization: Summary of an NHLBI working group. Transfusion. 2011;**51**:435-441

[142] Daniels G, Poole J, de Silva M, Callaghan T, MacLennan S, Smith N. The clinical significance of blood group antibodies. Transfusion Medicine. 2002;**12**:287-295

[143] Bayliss KM, Kueck BD, Johnson ST, Fueger JT, McFadden PW, Mikulski D, et al. Detecting fetomaternal hemorrhage: A comparison of five methods. Transfusion. 1991;**31**(4):303-307

[144] Kim YA, Makar RS. Detection of fetomaternal hemorrhage. American Journal of Hematology. 2012;**87**(4):417-423

[145] Pelikan DM, Mesker WE, Scherjon SA, Kanhai HH, Tanke HJ. Improvement of the Kleihauer-Betke test by automated detection of fetal erythrocytes in maternal blood. Cytometry. Part B, Clinical Cytometry. 2003;**54**(1):1-9

[146] Renshaw AA. An accurate and precise methodology for routine determination of the false-negative rate of Papanicolaou smears screening. Cancer. 2001;**93**(2):86-92

[147] Raafat A, Fraser N, Main R, Urbaniak SJ. A quality assurance scheme for the Kleihauer test: The Scottish experience 1988-1996. Transfusion Medicine. 1997;**7**:221-226

[148] Duguid JK, Bromilow IM. Laboratory measurement of fetomaternal hemorrhage and its clinical relevance. Transfusion Medicine Reviews. 1999;**13**:43-48

[149] Iyer R, McElhinney B, Heasley N. False positive Kleihauer tests and unnecessary administration of anti-D immunoglobulin. Clinical and Laboratory Haematology. 2003;**25**:405-408

[150] Chen JC, Bigelow N, Davis BH. Proposed flow cytometric reference method for the determination of erythroid F-cell counts. Cytometry. 2000;**42**:239-246

[151] Davis BH, Olsen S, Bigelow NC, Chen JC. Detection of foetal red cells in fetomaternal haemorrhage using a foetal haemoglobin monoclonal antibody by flow cytometry. Transfusion. 1998;**38**:749-756

[152] British Committee for Standards in Haematology (BCSH—b). Guidelines for the use of platelet transfusions. British Journal of Haematology. 2003;**122**:10-23

[153] Menitove JE. Immunoprophylaxis for D negative patients receiving platelet

transfusion from D negative donors. Transfusion. 2002;**42**:136-138

[154] Royal College of Obstetrics and Gynaecologists. The management of gestational trophoblastic disease. 2010

[155] Royal College of Obstetrics and Gynaecologists. The Use of Anti-D Immunoglobulin for Rhesus D Prophylaxis. 2011