Antibody Screening and Identification in Women of Child-bearing age in Zimbabwe.

[1]R Musonza, [2]MT Manase , [1]A Mandisodza,

1. Department of Medical Laboratory Sciences
2. Department of Obstetrics and Gynaecology

Correspondences: Prof Arthur Mandisodza, Department of Medical Laboratory Sciences, College of Health Sciences, University of Zimbabwe. PO Box A178, Avondale, Harare. Zimbabwe.

***Email*:** amandisodza@yahoo.com, **Phone:** +263 24791631: **Extn**: 2397/2295: **Mobile:** +263 772 249 569

1.0 Abstract

Background: Haemolytic disease of newborn is a haematological condition in which the foetal red blood cells are destroyed by the action of clinically significant alloantibodies from the mother through placental transfer leading to anaemia and jaundice. Maternal alloimmunization is well known to cause haemolytic disease of newborn. Maternal alloimmunization results in the production of antibodies by the mother after sensitization by incompatible foetal red cell antigens. The alloimmunization results from previous pregnancy, in utero haemorrhage or blood transfusion. The frequency of alloimmunization in women varies globally. The current study aimed at determining the frequency of alloimmunization in women of child-bearing ages in Zimbabwe.

Methods: A cross sectional clinical and laboratory based study was conducted between January 2017 and April 2017 on women of child-bearing age attending an Antenatal Clinic at Parirenyatwa Group of Hospitals. Blood samples were collected from 268 of these women and were analyzed at the Parirenyatwa Group of Hospitals Blood Bank Laboratory. Antibody screening and identification tests were done on the sera using 2-Vial Screen Cells from the National Blood Services Zimbabwe and 9-vial Identification Panel Cells from the South African National Blood Services.

Results: A total of 268 samples from women of child-bearing age attending Mbuya Nehanda Antenatal Clinic were collected and analyzed. A majority of 115 (42.9%) of the participants were in the age group of 26-30 years. The fewest were 16 (6%) and 15 (5.6%) in the 16-20 age group and over 36 years old respectively. One hundred and ninety- two (72%) and 19 (7%) of the participants had 2-3 and 4-5 previous pregnancies respectively. Seventy-three (27.2%) of the participants had previous history of obstetric complications and blood transfusion. Twenty-one (28.8%) of these had been previously transfused. Miscarriages were reported in 41 (56.2%) of them and 11(15%) confirmed having had infants with neonatal jaundice. All the samples tested negative after an immediate spin technique using both reagent *Screen Cells 1 and 2* following incubation at room temperature. Only 2 (0.7%) samples were strongly positive (**4+**) after incubation at 37^0C in antihuman globulin (AHG) and both were identified as anti-D.

Conclusion: It can be concluded that antibody screening and identification of anti-D was significant since it is the most common alloantibody that causes haemolytic disease of the newborn (HDN). However, Rhesus HDN is largely preventable by administration of

prophylactic anti-D. The prevalence of alloantibodies in women of childbearing age was within the expected range. Antibody screening and identification tests are critical to prevent HDN.

Key words: Alloimmunization, antibody screening, antibody identification, HDN, Prophylactic anti-D.

2.0 Introduction

Antibody screening and identification of alloantibodies are importation steps in safe blood transfusion practice and in the treatment and prevention of haemolytic disease of the newborn (HDN). Alloantibodies are antibodies that are produced by an individual who lacks a corresponding antigen. They can be immune or naturally occurring. An immune type occurs only after exposure to a known antigen. A naturally occurring type is present without known cause. It is always present in individuals who lack the corresponding antigen. It is thought its presence is associated with exposure, earlier in life, to bacterial material with similar antigenic structure. Anti-D in Rhesus negative and anti-AB in O individuals are good examples of immune and naturally occurring alloantibodies respectively *(Walker et al, 1990).*

Although blood transfusion is a relatively safe way of treating haematological defects, adverse effects, known as transfusion reactions, may occur from time to time. The majority of transfusion reactions often occur as a result of undetected alloantibodies in the recipient. These antibodies react with donor blood cells that may have corresponding antigens. Transfusion reactions may happen when recipient serum is not tested by the screening and identification procedures for alloantibodies that may react with donor blood *(Walker et al, 1990).*

HDN can result from alloimmunization of the pregnant woman by antigens of foetal origin, if the she lacks the same antigens. The antibodies produced by the woman are of the immune type and are usually of the IgG type. IgG has the capacity to cross the placenta and react with foetal red blood cells resulting in their destruction. Immunization of the mother by foetal red cells may occur during delivery, foeto-maternal haemorrhage or in utero foetal damage. Rhesus HDN due to anti-D in a Rhesus D negative mother is a good example and the most common type associated with alloimmunization. There are several blood group antigens whose corresponding alloantibodies can also cause this condition. HDN may also be caused by naturally occurring alloantibodies of the IgG type. These antibodies are always found in individuals without corresponding antigens and the cause of their presence is still not clearly understood *(Walker et al, 1990; Basu et al., 2011).*

The pathogenesis of HDN involves the passage of maternal antibodies of the IgG type across the placenta and the destruction (haemolysis) of foetal red blood cells by these antibodies, resulting in anaemia and jaundice. Haemolysis may lead to severe anaemia and an abnormal increase in fluids in vital organs, resulting in *Hydrops foetalis,* causing death. Although Rhesus HDN is very severe, it is preventable by giving the mothers prophylactic anti-D within a period of 72 hours after delivery. Anaemia in ABO HDN is mild due to weak foetal A or B antigens and presence of A or B substances in foetal circulation which may neutralize the anti-AB. Early detection of HDN has allowed treatment of ABO HDN through exchange transfusion, apheresis

and phototherapy. The destruction of haemoglobin causes hyperbilirubinaemia which results in *Kernicterus* with resultant brain damage *(Bricca et al., 2011; Cheng et al., 2012).*

However, problems may arise in situations where the mother already has immune or naturally occurring alloantibodies of the IgG type that are not detected through routine testing. Good examples of such antibodies are anti-E and anti-C^w, both of which are naturally occurring. These antibodies have potential to cause HDN *(Walker et al, 1990)*. Cases of HDN due to alloantibodies to other blood group antigens other than ABO and Rhesus D have been reported in a number of countries (*Semmekrot et al., 1999; Moise et al., 2000; Lurie et al., 2003*). The global variations in the alloimmunization of women of childbearing age were found to be very significant (*Filbey et al., 1995; Lee et al., 22003; Al-lbrahim et al., 2008*). Anti-E was found to be one of the most commonly detected antibodies of the IgG type in transfused persons (*Mandisodza et al., 2014*). If a pregnant woman is E negative, she is most likely to have an infant with HDN if the infant is E positive because anti-E is naturally occurring.

A retrospective study carried out in Zimbabwe showed prevalence of alloimmune antibodies to be within the expected range of 0.4% to 2.7% (*Cakana et al., 2000*).

Routinely, antibody testing in pregnant mothers during their antenatal clinic visits is done using the AHG test only to screen and identify anti- D. However, a recent study showed that HDN can also be caused by alloantibodies to other blood group antigens (*Sithole et al., 2017*).

Antenatal routine testing often involves only the ABO and Rhesus for the identification of A, B, AB and O blood groups and the Rhesus D status of the mother and infant. Presence of immune or naturally occurring alloantibodies of the IgG type can be detected by the screening and identification techniques. Once identified, and there is incompatibility between the mother and infant, appropriate treatment and prevention procedures for HDN are taken.

Due to current economic challenges, most women of childbearing age are not utilizing antenatal services, making them vulnerable to pregnancy related alloimmunization and HDN. Assuming they would have been immunized already through transfusion, alloimmune antibodies may be detected and identified for early prevention or management of HDN.

This study was carried out to screen and identify immune, especially anti-D, and naturally occurring alloantibodies in women of childbearing age. The study was aimed at determining the current prevalence of alloantibodies in this population.

3.0 Materials and Methods

3.1 Ethical Considerations

The study was carried out from 1 January 2017 to 30 April 2017, after approval was granted by the Joint Research Ethics Committee of the Parirenyatwa Group of Hospitals and University of Zimbabwe College of Health Sciences (**JREC Ref: 404/16**).

Permissions to collect blood samples and carry out the laboratory tests were granted by the Hospital Clinical Director and the Chief Medical Laboratory Scientist respectively.

The results and data of the participants were recorded and stored in a password protected device accessible to the researchers only and the information was used for research purposes

only. Maximum confidentiality of the participants was maintained throughout the study by assigning unique codes on all participant records.

3.2 Study design and study sites

This was a cross sectional clinical and laboratory based study carried out on women of child-bearing age attending Mbuya Nehanda Antenatal Clinic of the Parirenyatwa Group of Hospitals. Women of child-bearing age included those who were pregnant and those who had delivered. The study excluded women who had no previous pregnancy records. The women were approached sequentially on their routine visits to the antenatal clinic. The nature of the study was briefly explained to them, highlighting the risks and benefits involved. All those who were willing to participate and who met the inclusion criteria signed the informed consent forms.

3.3 Sample size determination

A study sample size of 196 was use based on the Dobson's formula:

$S=Z^2pq/E^2$, *where;*

Z = Test statistics

E = Standard Error

P = Population Proportion with desired characteristic

q = 1- p

S = minimum sample size

3.4 Sample Analysis

Antibody screening was performed on serum samples using two vials of reagent O red blood cells (*Screen Cell 1 and 2 -Lot SC 1001-17*). These consist of a combination of critical antigens such as D, C, E, c, e, M, N, S, s, U, P_1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a and Jk^b in order to detect the corresponding alloantibodies. The reagent cells were supplied by National Blood Service Zimbabwe (NBSZ), NBSZ Center in Bulawayo, Zimbabwe.

Antibody identification was performed on samples that were antibody screen positive using nine vials of reagent O red blood cells from the South African National Blood Service (SANBS) (*9-vial SANBS Reagent Panel Cells-Lot 127*). The reagent O red blood cells also consisted of a combination of known critical antigens such as D, C, E, c, e, M, N, S, s, U, P_1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a and Jk^b. The Anti-human Globulin (AHG) test was used for both techniques in order to detect and identify clinically significant alloantibodies which react best at 37^0C.

3.5 Data Analysis

Results and demographic data were captured on a Microsoft Excel spread sheet. Descriptive statistics were used to summarize the categorical and continuous data. Frequencies of alloantibodies detected and identified were calculated as percentages of the total number of women of child-bearing age. Some results were shown as tables drawn using Microsoft Excel.

4.0 Results

A total of 268 samples from women of child-bearing age attending Mbuya Nehanda Antenatal Clinic were collected and analyzed.

A majority of 115 (42.9%) of the participants was in the age group of 26-30 years. The fewest were 16 (6%) and group15 (5.6%) between 16-20 age and over 36 years old respectively (*Table I*).

One hundred and ninety- two (72%) and 19 (7%) of the participants had 2-3 and 4-5 previous pregnancies respectively (*Table I*).

Seventy-three (27.2%) of the participants had previous history of obstetric complications and blood transfusion. Twenty-one (28.8%) of these had previous history of blood transfusion. Forty-one (56.6%) had previous miscarriages and 11 (15.0%) confirmed having had infants with neonatal jaundice (*Table II*).

All the 268 samples tested negative after an immediate spin technique using both reagent *Screen Cells 1 and 2* that were incubated at room temperature. Antibody screening detected only 2 (0.7%) samples that were strongly positive (**4+**) after incubation at 37^0C in AHG and both were identified as anti-D.

Table I: ***Demography of Women of Child-bearing Age (n=268).***

Age group(years)	***N***	(%)
16-20	16	**6.0**
21-25	69	25.7
26-30	115	42.9
31-35	53	19.8
>36	15	5.6
TOTAL	**268**	**100**
Number of Previous Pregnancies		
0-1	57	21
2-3	192	72
4-5	19	7
TOTAL	**268**	**100**

Table II: ***History of Previous Transfusion and Obstetrics Complications (n=73)***

HISTORY	**N**	**(%)**
Previous transfusion	21	**28.8**
TOTAL (*Transfusions*)	**21**	**28.8**
Previous Miscarriages		
Once	29	**39.7**
Twice	8	**11**
Thrice	3	**4.1**
>Fourth	1	**1.4**
TOTAL ***(Miscarriages)***	**41**	**56.2**
History of neonatal jaundice	11	**15.0**
TOTAL (*Neonatal Jaundice****)***	**11**	**15.0**
TOTAL	**73**	**100**

5.0 Discussions and Conclusions

Two hundred and sixty-eight participants from women of child-bearing age were involved in the study. This was way above the calculated sample size (196) using the Dobson method. Therefore, the reliability of the results was not affected by sample size.

The majority of women of child-bearing age were young adults in the age group of 26-30 years. The age group falls within the recommended ages considered most secure for child bearing. There are poor outcomes for mothers who have pregnancy over the age of 35 years. Genetic abnormalities such as Down syndrome, and miscarriages have also been found to increase with births over this age (*Bewley et al., 2005*). On the other extreme, adolescence pregnancy was found to be associated with delays in seeking help resulting in poor health and social outcomes. Anaemia and poor nutrition were common in this age group. The anaemia resulted in complication in births. Adolescent pregnancy was also associated with poor obstetric outcomes such as hypertension, preterm labour and delivery, low birth weight and increased morbidity and mortality (*Dopkin-Boecker* and *Adams Hillard, 2009*).

All participants had history of previous pregnancy. Pregnancy is a major risk factor for alloimmunization in women of childbearing age. Alloimmunization occurs when a woman of childbearing age is negative for a specific red cell antigen and the infant is positive for the same

antigen the mother will produce an antibody against the foetal antigen. If the antibody is of the IgG type or IgA- monomer type, it will cross the placenta and react with foetal red cells and destroys them, resulting in HDN. Rhesus HDN is the most common of all alloimmune mediated HDNs, although it is largely preventable through the use of prophylactic anti-D. There are several non-Rhesus alloantibodies that have been implicated in causing HDN (*Walker et al., 1990; Geifman-Holtzman, 1997; Kennedy et al., 2017*). Studies have shown that even though anti-M was of the IgM type in the mother, there was a fraction of anti-M that was of the IgG type which caused HDN in her twin infants (*Arora et al., 2015*). Therefore, monitoring pregnancies by antibody screening and identification is critical for the prevention and treatment of HDN.

Although a small number of women of childbearing age were transfused, alloimmunization could occur if these women were given blood that was incompatible. That is, if an individual is negative for the antigen which is present in donor blood, he or she would most likely produce an antibody against the antigen. Presence of alloantibodies of the IgG type in the mother due to transfusion has the potential to cause HDN in infants that have corresponding antigens. Therefore, transfusion is one of the major causes of alloimmunization (*Walker et al., 1990*).

Previous miscarriages and neonatal jaundice were reported in the study. Neonatal jaundice, miscarriages and stillbirths are highly associated with HDN, especially non-Rhesus D because most of these are not tested routinely for alloimmunization (*Walker et al., 1990; Moise et al., 2000; Bouturao-Neto et al., 2006*).

In the current study, antibody screening and identification only detected anti-D. Alloimmunization by Rhesus D should have been prevented in the first place, if these women of childbearing age had been attending antenatal clinics for routine testing. The prevalence of alloantibodies was consistent with other findings which showed a 0.4% to 2.7% range (*Filbey et al., 1995; Al-lbrahim et al., 2008; Sidhu et al., 2016*). A similar study in Australia gave exactly the same results as those found in the current study in Zimbabwe, although the alloantibodies were variable with anti-E showing the highest prevalence followed by anti-D (*Manika and Bronwyn, 2015*). Anti-E is one the few naturally occurring antibodies of the Rhesus blood group system (*Walker et al., 1990*). Therefore, it was expected to be present in women of childbearing age who were E negative. Since twenty-two percent of blacks are E negative, one would expect the anti-E to have been detected and identified as a naturally occurring antibody in the study. Most studies have identified anti-E as the most common after blood transfusion and previous pregnancies (*Cakana* and *Ngwenya, 2000; Moran et al., 2000; Mandisodza et al., 2014*).

It can be concluded that antibody screening and identification of anti-D was significant because anti-D is the most common alloantibody associated with haemolytic disease of the newborn (HDN). However, Rhesus HDN is largely preventable by administration of prophylactic anti-D. Prevalence of alloantibodies in the study was within the range of results found in other studies. The absence of anti-E in the study was worrying because the antibody is naturally occurring and was expected to be present. Most studies have shown this to be the case.

It is strongly recommended that steps should be taken to facilitate tests to screen and identify alloantibodies with potential to cause HDN as a way of treating and managing this condition.

6. References

1. Al-lbrahim NA, Alsaeed AH, 2008. Red blood cell alloimmunization among Saudi pregnant women in the central province of Saudi Arabia. *Kuwait Medical Journal;* 40: 116-123.
2. Arora,S.,Doda, V., Maria,A., Kotwal, U and Goyal,S. (2015): Maternal anti-M induced hemolytic disease of newborn followed by prolonged anemia in newborn twins. *Asian Journal of Transfusion Science*, 9(1): 98-101.
3. Basu S, Kaur R, Kaur G, 2011. Hemolytic disease of the fetus and newborn: Current trends and perspectives. *Asian Journal of Transfusion Sciences*; **5**(1), 3-7.
4. Bewley S, Davies M, Braude P, 2005. The most secure age of childbearing remains 20-35 years. *British Medical Journal*; 33 (7517): 588-589.
5. Boturão-Neto E, Chiba AK, Barros MMO, _de Mello AB, Fabron Jr A, Bordin JO, 2006 Dec. Anti-KEL7 (anti-Js^{b}) alloimmunization diagnostic supported by molecular *KEL∗6,7* typing in a pregnant woman with previous intrauterine deaths. *Transfusion and Apheresis Science;* 35 (3): 217-221
6. Bricca P, Guinchard E, Guitton BC, 2011. Management of foeto-maternal red cell allo-immunizations. *Transfusion Clinical Biology Journal Society Francaise Transfus Sang*; 18 (2): 269-276.
7. Cakana AZ and Ngwenya L, 2000. Is antenatal antibody screening worthwhile in the Zimbabwean population? *Central African Journal Medicine*, 46 (2):38–41.
8. Cheng CK, Lee CK, Lin CK, 2012. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese Thalassaemia Major patients and literature review. *Transfusion;* 52(10): 2220-2224.
9. Dopkin Broecker JE, Adams Hillard PJ, 2009. Pregnancy in Adolescence. *Global Library of Women's Medicine; DOI.10.3843/GLOWM.10414*
10. Filbey D, Hanson U, Wesström G, 1995. The prevalence of red cell antibodies in pregnancy correlated to the outcome of the newborn: A 12 year study in central Sweden. *Acta Obstetrics Gynecolology Scan*d; 74: 687–692.
11. Geifman-Holtzman WM, Kosmas AR, 1997. Female alloimmunization with antibodies known to cause hemolytic disease. *Obstetric Gynecology;* 89 (2)**:** 272–275.
12. Kennedy MS, Moise Jr KJ, Jul 2017. Management of non-Rhesus (D) red blood cell alloantibodies during pregnancy. *UpToDate*; Peer review process.
13. Lee CK, Ma, ESK, Tang M, Lam CCK, Lin CK, Chan LC. 2003. Prevalence and specificity of clinically significant red cell alloantibodies in Chinese women during pregnancy: A review of cases from 1997 to 2001. *Transfusion Medicine Oxford England*; 13 (4): 227–231.

14. Lurie S, Eliezer E, Piper I, Woliovitch I, 2003. Is antibody screening in Rh (D)-positive pregnant women necessary? *The Journal of Maternal Fetal and Neonatal Medicin*e, 14 (6): 404-406.
15. Mandisodza, AR, Hove P, Marques DS, Mberi E, Maramba, A, Dandavare C *et al*., 2014: Antibodies identified in transfusion dependent patients in Zimbabwe. *Journal of Applied Science in Southern Africa*, 20 (2): 45-51.
16. Manika P, Bronwyn W, 2015. Prevalence of maternal red cell alloimmunization: A population study from Queensland, Australia. *The Journal of Royal College of Australasia*; 47 (2):151–155.
17. Moise JK, 2000. Non-anti-D antibodies in red-cell alloimmunization. *European Journal of Obstetrics and Gynecology and Reproductive Biology;* 92**:** 75-81.
18. Moran P, Robson SC, Reid MM, 2000. Ant-E in Pregnancy. *British Journal of Obstetrics and Gynaecology;* 107 (11): 1436-1438.
19. Semmekrot BA, de Man AJ, Boekkooi PF, van, Dilk BA, 1999. Irregular blood group antibodies during pregnancy: Screening is mandatory. *Ned Tijdschr Geneeskd*, 143(28): 1449–1452.
20. Sidhu M, Bala R, Akhtar N, Sawhney V, 2016. Prevalence, Specificity and Titration of Red Cell Alloantibodies in Multiparous Antenatal Females at a Tertiary Care Centre from North India. *Indian Journal Hematology and Blood Transfusion of the Indian Soc Hematology Blood Transfusion*; 32 (3): 307–311.
21. Sithole S, Mandisodza A, Maramba A, Chikwasha V, Magwali T, Mavunganidze G, 2017. Inadequate uptake of prophylactic anti-D to prevent Rhesus haemolytic disease of the foetus or newborn in Zimbabwe. *Central African Journal of Medicine*; In Press.
22. Walker RH, Hoppe PA, Judd WJ, Ness P, Polesky HF, Rolih SD *et al*., 1990. Transfusion Practice and Adverse effects of Blood Transfusion. *Technical Manual; American Association of Blood Banks;* 10th Edition: 173-290, 341-429, 381-386, 393-395.