Chapter 6

MATERNAL RED BLOOD CELL ALLOIMMUNIZATION IN SOUTH WESTERN UGANDA

Bernard Natukunda, Godfrey Mugyenyi, Anneke Brand and Henk Schonewille. Maternal red blood cell alloimmunisation in South Western Uganda. *Transfusion Medicine*, 2011, 21, 262–266

ABSTRACT

Objectives: To determine the prevalence of RhD negativity and the rate of red blood cell (RBC) alloimmunization in Ugandan pregnant women.

Aim: To identify the frequency and nature of maternal RBC alloimmunization in Uganda.

Background: Haemolytic disease of the fetus and newborn (HDFN) results from maternal alloimmunization following exposure to allogeneic RBCs during pregnancy or blood transfusion. The prevalence of maternal RBC alloimmunization in Ugandans is not known.

Materials and methods: Pregnant women at Mbarara Hospital, South Western Uganda, were investigated in a cross-sectional study. Demographics, transfusion and obstetric histories were recorded. Maternal RBC alloimmunization was demonstrated using immunohaematological techniques.

Results: A total of 2001 pregnant women were recruited; 3.6% of them being RhD-negative. Forty five women (2.2%; 95% CI: 1.6-2.9) were found to be alloimmunized to RBC antigens. There were 38 RBC alloantibodies of known specificity including: anti-S, 12; anti-M, 11; anti-Le^a, 6; anti-D, 4; and 1 each of anti-K, -Fy^b, -Jk^a, -Lu^a , and -Kp^a. In two women (4.4%), there were antibody combinations (anti-M+S and -K+Kp^a). Obstetric history, gestational age and previous immunizing events were not significantly associated with the rate of alloimmunization.

Conclusions: This study revealed a maternal RBC alloimmunization rate of 2.2% which was comparable with findings from a Zimbabwean study where the prevalence was 1.7%. Given the 6.0% prevalence of anti-D among RhD-negative women in our study and the high immunogenicity of the D antigen, programs for preventing anti-D alloimmunization and HDFN in Uganda should be considered seriously.

Key words: Maternal alloimmunization, RBC alloantibodies, Pregnancy, Haemolytic disease of the newborn, Uganda.

INTRODUCTION

Rhesus sensitization was first reported in Africans by Zoutendyk in 1947 when he described three cases in South African Bantu (Jacob *et al.,* 1959). Maternal alloimmunization is defined as the presence of irregular red blood cell (RBC) alloantibodies in the blood of a pregnant woman that can theoretically cause haemolytic disease of the fetus and newborn (HDFN). Virtually all alloantibodies reactive by the indirect antiglobulin test (IAT) have been implicated in HDFN in different populations. Most severe HDFN associated with intrauterine death is reported in women with Rh-D, -c and K alloantibodies (van Kamp *et al.,* 2004; Moise, 2008, Koelewijn *et al.,* 2008). In Caucasians, the D antigen accounts for about 50% of cases of maternal alloimmunization; the remainder is mainly due to incompatibility to K, c, C/G, E, and Fy^a antigens and to low incidence antigens in the Rh, MNS, and Diego blood group systems (Heddle *et al.,* 1993; Moise, 2008, Koelewijn *et al.,* 2008). Before the introduction of anti-D immunoprophylaxis, HDFN due to anti-D affected approximately 1% of all newborns and was responsible for the death of 1 baby in every 2200 births in developed countries (Kumar & Reagan, 2005).

The prevalence of D-negativity varies in different ethnic groups with approximately 15% of Caucasians, 8% of Blacks and 1% of Asians being D-negative (Reid & Lomas-Francis, 2004). A retrospective study in Zimbabwe showed that 191 (0.85%) of 22,493 infants had HDFN; 25 (13.1%) and 163 (85.3%) of these having D- and ABO-HDFN respectively (Mandisodza *et al.,* 2008). However, in Zimbabwe only 3.3% of the population is D negative and anti-D prophylaxis is routinely available (Cakana & Ngwenya, 2000). In a recent study, the prevalence of RhD negative patients at Mbarara Regional Referral Hospital (MRRH) in Mbarara, Uganda, was found to be approximately 6.0% (Natukunda & Smit Sibinga, unpublished observations). No anti-D prophylaxis is provided at MRRH and other public hospitals in Uganda. The prevalence of maternal alloimmunization due to RhD and other RBC antigens in Ugandan women is not known. The aim of this study was to provide data on the frequency and nature of maternal RBC alloimmunization in pregnant women in South Western Uganda. The findings from this study might be of relevance in planning future management strategies for HDFN in Uganda.

STUDY DESIGN AND METHODS

Study participants

In a cross-sectional study, pregnant women attending the antenatal clinic and those in labour at Mbarara Regional Referral Hospital, Mbarara, Uganda, were consecutively enrolled between March 1st and May 31st, 2010. Informed consent was obtained from all participants. The demographic characteristics, obstetric and transfusion histories were recorded in a data collection form. Information regarding the ABO and RhD blood groups of the participants was retrieved from their antenatal cards whenever available. The study was approved by the research and ethics committees at Mbarara University of Science and Technology, Mbarara, Uganda.

Laboratory investigations

After consent, 4ml of whole blood was drawn from each participant and put in ethylenediaminetetraacetic acid (EDTA) tubes for laboratory investigations. Plasma samples were removed and stored frozen at -80˚C, at the Epicentre Mbarara Research Base, until they were shipped to the Sanquin Blood Supply in Leiden, the Netherlands for immunohaematological studies. The samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs. In the indirect antiglobulin test (IAT), a LISS-enhanced gel centrifugation technique (DiaMed ID, Micro Typing System, Cressier sur Morat, Switzerland) with polyspecific antihuman globulin (rabbit anti-IgG and monoclonal anti-C3d) was used. When the antibody screening was positive, antibody identification was performed by testing the plasma samples with commercial panels of reagent RBCs, of selected phenotypes, by similar or additional methods whenever needed. Participants were considered to be alloimmunized if antibodies to one or more RBC antigens could be identified.

Statistical methods

Statistical software packages (Excel 5.0, Microsoft, Redmond, WA; and Statistical Package for the Social Sciences 12.0, SPSS, Inc., Chicago, IL) were used for data management and analysis, respectively. For univariate analysis of possible associations between maternal RBC alloimmunization and age at the time of enrolment, gestational age, parity, history of blood transfusion, previous Caesarean deliveries, and a history of antepartum haemorrhage, the Chi-

square test or Fisher's exact test were used. Groups were assumed to differ significantly when the probability level was less than 0.05.

RESULTS

Patient data

We recruited a total of 2001 pregnant women at Mbarara Regional Referral Hospital during the 3-month study period. Of these, 717 (35.8%) were in labour and admitted to the maternity ward while the others (n=1284) were outpatients attending the antenatal clinic with a mean gestational age of 27.2 (median, 28; range, 10 - 42) weeks. The mean age at the time of enrolment was 25.1 (median, 24; range, 14 - 46) years. The mean parity was 2.6 (median, 2; range, 1-12) with 687 (34.3%) women being primigravidae. Of 1881 women typed for their RhD status, 67 (3.6%) were RhD negative.

Table 1: RBC alloimmunization and immunizing events among 2001 pregnant women in different age groups at Mbarara Regional Referral Hospital in Mbarara, Uganda

	Maternal age (years)			Total
	14 - 19	20 - 35	>35	
Number of women (n)	263	1634	104	2001
Parity (median, range)	1 (1-4)	2 (1-12)	6 (1-12)	2 (1-12)
History of immunizing event[1]	4 (1.5)	307 (18.8)	40 (38.5)	351 (17.5)
Alloimmunized women	10 (3.8)	30 (1.8)	5 (4.8)	45 (2.2)
Number of antibodies (n)	11	30	6	47
Women with panreactive or aspecific antibodies	3 (1.1)	4 (0.2)	2 (1.9)	9 (0.4)
Women with clinically significant antibodies	5 (1.9)	21 (1.3)	3 (2.9)	29 (1.4)
Clinically significant antibodies (n)	6	21	4	31
Anti-S	2	9	1	12
Anti-M	2	9	0	11
Anti-D	1	3	0	4
Anti-K, -Fy^b, -Jk^a or -Kp^a,	1	1	2	4

Data presented as number (%) unless stated otherwise. [1] Caesarean delivery, antepartum haemorrhage, blood transfusion

In the obstetric history, 186 (9.3%) participants reported having delivered by Caesarean section and 14 (7.5%) of them had received a blood transfusion; 159 (7.9%) participants had experienced a prior antepartum haemorrhage (APH) of whom 59 (37.1%) had also been transfused. A history of blood transfusion for non-obstetric indications was recalled by 5 women. Overall, 78 participants (3.9%) gave a history of past exposure to blood transfusion.

Table 2: Demographic variables, transfusion history and obstetric characteristics of RBC immunized and non-immunized pregnant women at Mbarara Regional Referral Hospital in Mbarara, Uganda[1]

Demographics	Immunized women (n=45)	Women with clinically relevant antibodies (n=29)	Non-immunized women (n=1956)
Age in years	24.9 (24; 17-37)	25.1 (24; 17-37)	25.1 (24; 14-46)
Gestational age ≤28 weeks	18 (2.4)[2]	13 (1.8)[2]	715 (36.6)[3]
Parity:			
Primiparae	20 (2.9)	13 (1.9)	667 (34.1)
Para 2 - 5	23 (2.0)	15 (1.3)	1135 (58.0)
Para >5	2 (1.3)	1 (0.6)	154 (7.9)
History of sensitizing events	8 (2.3)	6 (1.7)	343 (17.5)
Multiple sensitizing events[4]	1 (1.4)	0	72 (3.7)
No sensitizing events	37 (2.2)	23 (1.4)	1613 (82.5)

[1] Data are reported as mean (median; range) or number (percentage). [2] Percentage per row calculated with total number of immunized and non-immunized women for: gestational age ≤28 weeks, parity, history of sensitizing events, multiple sensitizing events and no sensitizing events as the denominator; respectively.
[3] Percentage per row calculated with total number of non-immunized women as the denominator.
[4] Caesarean delivery and blood transfusion or antepartum haemorrhage and blood transfusion.

Maternal RBC alloimmunization

Forty five women (2.2%; 95% CI: 1.6-2.9) were found to be alloimmunized to RBC antigens; 20 (44.4%) of them being primigravidae. Only 1 (2.2%) of the alloimmunized women recalled a history of previous blood transfusion. The proportions of alloimmunized women and the antibodies relevant for HDFN in different age groups are shown in Table 1. Maternal age (<20, 20-35 and >35 years), parity (primiparae, para 2-5 and para >5), past history of sensitizing events (Caesarean deliveries and APH with or without blood transfusions, blood transfusions for non-obstetric indications) and the gestational age (first, second or third trimester) at enrolment were

neither significantly associated with the overall rate of alloimmunization nor with the rate of formation of clinically significant alloantibodies ($p>0.2$ for all). Table 2 summarizes the demographic variables, transfusion history and obstetric characteristics of the 2001 pregnant women studied at Mbarara Regional Referral Hospital.

RBC antibody specificities

There were 38 RBC alloantibodies of known specificity produced by 36 of the alloimmunized women. The remaining 9 women possessed non-specific (n=6) or pan-reactive antibodies (n=3). The alloantibody specificities identified were: anti-S, 12; anti-M, 11; anti- Le^a, 6; anti-D, 4; and 1 each of anti-Fy^b, -K, -Jk^a, -Lu^a , and -Kp^a. These presented as antibody combinations of anti-M+S and anti-K+ Kp^a in two of the women (4.4%); the remaining of the identified alloantibodies were as single specificities.

DISCUSSION

In this cross-sectional study, 45 out of 2001 Ugandan pregnant women had RBC alloantibodies giving an overall maternal alloimmunization rate of 2.2% (95% CI: 1.6 - 2.9). Out of 47 alloantibodies detected, 31 antibodies (66.0%) in 29 women (1.4%) can be considered clinically relevant with reported potential to cause HDFN (Daniels, 2002). These included 4 anti-D, 12 anti-S, 11 anti-M, and 1 each of anti-K, -Fy^b, -Jk^a, and -Kp^a. A limitation of our study was the inability to determine the RBC antigens from newborns, to confirm the paternal alloantigen origin. We presume, however, that most of these alloantibodies were formed against paternally derived fetal RBC antigens since none of the alloimmunized women with clinically significant antibodies reported a history of prior blood transfusion, although anti-M is known for its occurrence as a natural antibody. The overall prevalence of maternal RBC alloimmunization is comparable with findings from a study in Zimbabwe by Cakana *et al*. (2000) in which 50 out of 3000 pregnant women (1.7%) had RBC antibodies. In this study, however, only seven women (0.2%) possessed antibodies clinically significant for HDFN (i.e. 4 anti-D, 2 anti-E, and 1 anti-Js^b). Recently, Belinga *et al*. (2009) reported a higher prevalence of maternal RBC alloimmunization in 15 of 225 (6.7%) Cameroonian women and of these, 9 women (4.0%) possessed clinically relevant RBC alloantibody specificities (i.e. anti-D).

Maternal age, parity, history of sensitizing events and gestational age were not significantly associated with the rate of alloimmunization. In a Dutch study, previous RBC transfusion was the most important risk factor for non-D alloimmunization during pregnancy (Koelewijn *et al.,* 2009). We previously showed that severe anaemia due to malaria was the indication for transfusion in 39% of Ugandan blood recipients and that 83% of them were young children (Natukunda *et al.,* 2010a). It is possible that not all the women in the current study could recall being transfused in early childhood, which may explain the absence of previous transfusion as a risk factor for maternal alloimmunization. Since parity increases with age, the clinically relevant RBC alloimmunization rate might also rise with maternal age. However, the alloimmunization frequency in our teenage pregnant women was comparable to the older women (p=0.7) and remarkably, primiparae showed the highest immunization rate. One could speculate that persistence of an antibody formed after an unrecognized transfusion during childhood explains antibody prevalence in the young women, while a high parity is responsible for antibody prevalence in the older women. Transfusion recipients in Uganda are more likely to become alloimmunized because of substandard pre-transfusion practices in some clinical and laboratory settings. Notably, we previously observed nulliparous sickle cell patients who produced anti-D post-transfusion despite a local transfusion policy of matching for the RhD antigen, suggesting RBC typing errors (Natukunda *et al.,* 2010b).

Anti-S alloantibodies were the most frequent RBC antibody specificity found. We have previously reported a high frequency of anti-S alloimmunization following blood transfusion in the Ugandan population as well (Natukunda *et al.,* 2010b; Natukunda *et al.,* 2010c). Therefore, anti-S may be considered for future studies in S-negative pregnant women to evaluate the associated incidence of (severe) HDFN. Anti-M was the second most frequent antibody. Due to the fact that low titer anti-M alloantibodies are rarely implicated in HDFN (Koelewijn *et al.,* 2008; Wikman *et al.*, 2007), and case reports suggest that HDFN is restricted to titers above 128, we carried out titrations for this specificity in 9 samples for which sufficient plasma was available. The titers were $\leq$32 in all the samples (data not shown). Therefore, the clinical significance of anti-M as a cause of HDFN in Ugandan women is not likely.

The frequency of anti-D immunization among RhD negative women was 6.0%. Of the four women with anti-D, three were multigravidae (gravida 2-6) while the fourth one was a primegravida at 26 weeks of gestation and she had no history of prior sensitizing events. One of

the multigravidae women with anti-D alloimmunization was in her sixth pregnancy and she also had a history of prior Caesarean delivery. The anti-D alloimmunization frequency herein reported is comparable to that in Caucasians before the introduction of Rh immune globulin (RhIG) prophylaxis (Woodrow & Donohue, 1968). Therefore, programs for prevention of maternal anti-D alloimmunization might be put in place in Uganda given the high immunogenicity of the D antigen. Although pregnant women are routinely tested for ABO and RhD blood groups during the antenatal booking visit, they are currently not screened for the presence of RBC alloantibodies. We recommend that all RhD negative pregnant women should be screened for alloanti-D. This will help to identify those RhD negative women who require anti-D prophylaxis, in particular within 72 hours postpartum when an RhD positive baby has been delivered. In the current study this policy might, in theory, have prevented anti-D in three out of the four cases. Challenges associated with implementation of this policy in Uganda include lack of constant availability of anti-D immunoglobulin, insufficient reagents for alloantibody screening, and absence of laboratory facilities for estimation of FMH – the Kleihauer-Betke acid elution technique (Kleihauer *et al.,* 1957; BCSH Guidelines, 1999) or flow cytometry (Nance *et al.,* 1989; Nelson *et al.,* 1998) – in order to determine the correct dosage for RhIG immunoprophylaxis. For those RhD-negative mothers who are found to be already alloimmunized, they should be followed up serologically and management strategies for safe delivery of the baby are required (Bowman, 1997), the lack of facilities and expertise for intrauterine transfusions notwithstanding. The affected neonate might then benefit from intensive phototherapy and exchange transfusions (Gottstein & Cooke, 2003).

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