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SHORT COMMUNICATION

Maternal red blood cell alloimmunisation in South Western Uganda

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SUMMARY

Aims/Objectives: To identify the frequency and nature of maternal red blood cell (RBC) alloimmunisation in Uganda and to determine the prevalence of RhD negativity and the rate of RBC alloimmunisation in Ugandan pregnant women.

Background: Haemolytic disease of the foetus and newborn (HDFN) results from maternal alloimmunisation following exposure to allogeneic RBCs during pregnancy or blood transfusion. The prevalence of maternal RBC alloimmunisation 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 alloimmunisation 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 alloimmunised 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, anti-Fy^b, anti-Jk^a, anti-Lu^a and anti-Kp^a. In two women (4·4%), there were antibody combinations (anti-M+S and anti-K+Kp^a). Obstetric history, gestational age and previous immunising events were not significantly associated with the rate of alloimmunisation.

Conclusions: This study revealed a maternal RBC alloimmunisation 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, programmes for preventing anti-D alloimmunisation and HDFN in Uganda should be considered seriously.

Key words: haemolytic disease of the newborn, maternal alloimmunisation, pregnancy, RBC alloantibodies, Uganda.

Rhesus sensitisation was first reported in Africans by Zoutendyk in 1947 when he described three cases in South African Bantu (Jacob *et al*., 1959). Maternal alloimmunisation 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 foetus 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, Rh-c and Rh-K alloantibodies (van Kamp *et al*., 2004; Koelewijn *et al*., 2008; Moise, 2008). In Caucasians, the D antigen accounts for about 50% of cases of maternal alloimmunisation; 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; Koelewijn *et al*., 2008; Moise, 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 one baby in every 2200 births in developed countries (Kumar & Regan, 2005).

The prevalence of D negativity varies in different ethnic groups with approximately 15% of Caucasians,

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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 alloimmunisation 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 alloimmunisation 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 MRRH, Mbarara, Uganda, were consecutively enrolled between 1 March 2010 and 31 May 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, 4 mL of whole blood was drawn from each participant and put in ethylenediaminetetraacetic acid 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 three-cell panel of reagent group O RBCs. In the 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 alloimmunised if antibodies to one or more RBC antigens could be identified.

Statistical methods

Statistical software packages (EXCEL 5.0, Microsoft, Redmond, WA, USA and STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES 12.0, SPSS, Inc., Chicago, IL, USA) were used for data management and analysis, respectively. For univariate analysis of possible associations between maternal RBC alloimmunisation and age at the time of enrolment, gestational age, parity, history of blood transfusion, previous Caesarean deliveries and a history of antepartum haemorrhage (APH), the Chi-squared 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 MRRH 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. 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 APH of whom 59 (37·1%) had also been transfused. A history of blood transfusion for non-obstetric indications was recalled by five women. Overall, 78 participants (3·9%) gave a history of past exposure to blood transfusion.

Maternal RBC alloimmunisation

Forty-five women (2·2%; 95% CI: 1·6–2·9) were found to be alloimmunised to RBC antigens; 20 (44·4%) of them being primigravidae. Only 1 (2·2%) of the alloimmunised women recalled a history of previous blood transfusion. The proportions of alloimmunised women and the antibodies relevant for HDFN in different age

Table 1. RBC alloimmunisation and immunising events among 2001 pregnant women in different age groups at MRRH in Mbarara, Uganda

	Maternal age (years)			
	14–19	20–35	>35	Total
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 immunising event[1] (*n*, %)	4 (1·5)	307 (18·8)	40 (38·5)	351 (17·5)
Alloimmunised women (*n*, %)	10 (3·8)	30 (1·8)	5 (4·8)	45 (2·2)
Number of antibodies (*n*)	11	30	6	47
Women with pan-reactive or aspecific antibodies (*n*, %)	3 (1·1)	4 (0·2)	2 (1·9)	9 (0·4)
Women with clinically significant antibodies (*n*, %)	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, anti-Fy^b, anti-Jk^a or anti-Kp^a	1	1	2	4

[1]Caesarean delivery, APH, blood transfusion.

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 sensitising 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 alloimmunisation nor with the rate of formation of clinically significant alloantibodies ($P > 0{\cdot}2$ for all). Table 2 summarises the demographic variables, transfusion history and obstetric characteristics of the 2001 pregnant women studied at MRRH.

RBC antibody specificities

There were 38 RBC alloantibodies of known specificity produced by 36 of the alloimmunised women. The remaining nine women possessed nonspecific ($n = 6$) 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, anti-K, anti-Jk^a, anti-Lu^a and anti-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 of 2001 Ugandan pregnant women had RBC alloantibodies giving an overall maternal alloimmunisation rate of 2·2% (95% CI: 1·6–2·9). Of the 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, anti-Fy^b, anti-Jk^a and anti-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 foetal RBC antigens as none of the alloimmunised 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 alloimmunisation is comparable with findings from a study in Zimbabwe by Cakana & Ngwenya (2000) in which 50 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 alloimmunisation in 15 of 225 (6·7%) Cameroonian women and of these, nine women (4·0%) possessed clinically relevant RBC alloantibody specificities (i.e. anti-D).

Maternal age, parity, history of sensitising events and gestational age were not significantly associated with the rate of alloimmunisation. In a Dutch study, previous RBC transfusion was the most important risk factor for non-D alloimmunisation 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 this study could recall being transfused in early childhood, which may explain the absence of previous transfusion as a risk factor for maternal alloimmunisation. As parity increases with

Table 2. Demographic variables, transfusion history and obstetric characteristics of RBC immunised and non-immunised pregnant women at MRRH in Mbarara, Uganda[1]

Demographics	Immunised women ($n = 45$)	Women with clinically relevant antibodies ($n = 29$)	Non-immunised 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 sensitising events	8 (2·3)	6 (1·7)	343 (17·5)
Multiple sensitising events[4]	1 (1·4)	0	72 (3·7)
No sensitising 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 immunised and non-immunised women for gestational age ≤28 weeks, parity, history of sensitising events, multiple sensitising events and no sensitising events as the denominator, respectively.
[3]Percentage per row calculated with total number of non-immunised women as the denominator.
[4]Caesarean delivery and blood transfusion or APH and blood transfusion.

age, the clinically relevant RBC alloimmunisation rate might also rise with maternal age. However, the alloimmunisation frequency in our teenage pregnant women was comparable to the older women ($P = 0·7$) and remarkably, primiparae showed the highest immunisation rate. One could speculate that persistence of an antibody formed after an unrecognised 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 alloimmunised 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 alloimmunisation following blood transfusion in the Ugandan population as well (Natukunda *et al*., 2010b,c). 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 titre anti-M alloantibodies are rarely implicated in HDFN (Wikman *et al*., 2007; Koelewijn *et al*., 2008), and case reports suggest that HDFN is restricted to titres above 128, we carried out titrations for this specificity in nine samples for which sufficient plasma was available. The titres were ≤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 immunisation 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 sensitising events. One of the multigravidae women with anti-D alloimmunisation was in her sixth pregnancy and she also had a history of prior Caesarean delivery. The anti-D alloimmunisation frequency herein reported is comparable to that in Caucasians before the introduction of Rh immune globulin (RhIG) prophylaxis (Woodrow & Donohue, 1968). Therefore, programmes for prevention of maternal anti-D alloimmunisation 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 h postpartum when an RhD-positive baby has been delivered. In this study this policy might, in theory, have prevented anti-D in three 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 fetomaternal haemorrhage (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 alloimmunised, 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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Bernard Natukunda, Anneke Brand and Henk Schonewille designed the research study, Bernard Natukunda and Godfrey Mugyenyi performed the research. Bernard Natukunda and Henk Schonewille analysed the data. Bernard Natukunda wrote the paper, which was edited by Anneke Brand and Henk Schonewille.

CONFLICT OF INTEREST

The authors certify that they have no affiliation with or financial involvement in any organisation or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

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