Chapter 4

RED BLOOD CELL ALLOIMMUNIZATION IN SICKLE CELL DISEASE PATIENTS IN UGANDA

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ABSTRACT

BACKGROUND: Blood transfusion is an integral part in the management of sickle cell disease (SCD) patients. Alloimmunization is a recognized complication of red blood cell (RBC) transfusions with consequences including delayed hemolytic transfusion reactions and difficulties in getting compatible blood for future transfusions. The objective of this study was to determine the frequency of RBC alloimmunization in SCD patients in Uganda where pre-transfusion screening for alloantibodies is not practiced.

STUDY DESIGN AND METHODS: In a cross-sectional study, SCD patients at Mulago Hospital Sickle Cell Clinic, Kampala, Uganda, were investigated. The demographic characteristics and transfusion history were recorded. Blood samples were drawn from consenting, previously transfused patients and RBC alloimmunization was demonstrated using immunohematological techniques.

RESULTS: There were 428 patients (median age, 12; F/M ratio, 1.0) and they had received a median 3 units in a median 3 transfusion episodes. Twenty six patients (6.1%) possessed RBC alloantibodies and 21 (80.7%) of them had received up to 10 transfusions. A total of 30 alloantibodies was found; 20 (66.7%) and 5 (16.6%) belonged to Rh and MNS blood groups respectively. Five of the alloimmunized patients had multiple antibodies.

CONCLUSION: The rate of RBC alloimmunization in Ugandan SCD patients was 6.1%. The homogeneity between donors and SCD patients plus the low transfusion load may explain this immunization frequency. Nevertheless, our study confirms the significance of RBC alloimmunization as a complication in Ugandan SCD patients. Therefore, there is need to improve immunohematological testing in Uganda so that RBC alloimmunization and its consequences may be prevented.

Keywords: Alloimmunization, Sickle cell disease, Red blood cell, Uganda.

INTRODUCTION

Sickle Cell Disease (SCD) is the most common genetic disease in Uganda where about 5 million people (20% of the total population) have the sickle cell trait and about 25,000 children are born with the disease each year.[1] Blood transfusion is an important therapeutic tool in the management of SCD: it increases the oxygen carrying capacity of the blood by increasing the hemoglobin concentration and decreasing the percentage of sickle hemoglobin by dilution.[2] However, red blood cell (RBC) alloimmunization is one of the complications of allogeneic blood transfusions and in general the risk increases with the number of blood transfusions, although many patients become alloimmunized early during transfusion therapy.[3] Various frequencies of RBC alloimmunization in SCD patients ranging from 2.6-76% have been reported in a number of studies.[4 – 16]

Alloimmunization may limit the availability of compatible blood for future transfusions and can contribute to perinatal morbidity due to hemolytic disease of the newborn.[17] In addition, delayed hemolytic transfusion reactions can mimic a sickle cell crisis and may be responsible for major morbidity in the SCD patient.[10, 16, 18] In the Cooperative Study of Sickle Cell Disease in the United States, multiple antibodies were detected in over 50% of alloimmunized subjects.[8] With time, many of the antibodies become undetectable, potentially confounding future transfusions and placing the patient at risk of anamnestic antibody production and delayed hemolytic transfusion reactions.[2, 19] The antigens most frequently involved belong to the Rh, Kell, Kidd, Duffy, Lewis and MNS blood group systems.[7, 8, 10, 11, 13, 20 - 22] Factors implicated in RBC alloantibody formation include recipient gender and age, history of pregnancy, number and timing of blood transfusions, recipient clinical diagnosis and treatment, genetic factors related to the antigenic response, and racial differences between donors and recipients.[7, 8, 20, 23 - 25]

The prevalence of post transfusion alloimmunization in Uganda, where blood donors and SCD patients are more racially homogeneous and where pretransfusion testing is only limited to ABO/D grouping plus a room temperature saline cross-match, is not known. The objective of this study was to determine the frequency and nature of RBC alloimmunization in SCD patients in Uganda.

MATERIALS AND METHODS

Patients

In a cross-sectional study, patients with homozygous SCD attending the Sickle Cell Clinic (SCC) at Mulago National Referral Hospital in Kampala, Uganda, were investigated. The study took place between 1st February and 31st July, 2008. Informed consent was obtained from the patients or their parents/guardians. Eligibility criteria included SCD patients who were at least 2 years of age and had received at least 2 previous allogeneic blood transfusions – the last transfusion episode being longer than 2 weeks before enrollment into the study. These criteria were chosen so as to study a group of patients that were most likely to have become alloimmunized at an appropriate age and time after exposures to RBC antigens. In general, patients received packed RBC transfusions compatible with their ABO and D phenotypes and which were not leukoreduced.

Data collection

Records at the SCC regarding the recruited SCD patients were reviewed for their demographic characteristics and the transfusion history. In cases of incomplete or missing records, older patients or accompanying parents and relatives were asked for additional information on the above history. The number of transfusion episodes, number of units of blood transfused, date of transfusion, indication for transfusion, age of first transfusion and a history of pregnancy were recorded in a data collection form. For patients who were first transfused in childhood and could not recall their exact age at first transfusion, we entered the age of *3 years* in the database for analysis. The study was approved by the research and ethical committees at Mbarara University of Science and Technology and Makerere University Medical School.

Laboratory investigations

After consent, blood was drawn for laboratory investigations. Frozen plasma and buffy coat samples were shipped to the Sanquin Blood Bank in Leiden, The Netherlands, for immunohematological studies. The plasma samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs. For 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. Patients were considered to be alloimmunized if antibodies to one or more RBC antigens were identified. DNA was extracted from buffy coat samples (using the QIAamp DNA Blood Midi Kit, Qiagen) of patients who possessed anti-D alloantibodies and D genotyping using an *RHD* multiplex polymerase chain reaction (PCR) was performed as described by Maaskant-van Wijk and co-workers.[26]

Statistical methods

Statistical software packages Excel 5.0 (Microsoft, Redmond, CA, 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 alloimmunization and age at the time of enrollment, age at first transfusion, gender, pregnancy history, number of transfusion episodes, number of units received and the indication for transfusion, the Chi-square test or Fisher's exact test were used for discrete variables. Logistic regression analysis was used for continuous variables of a non-Gaussian distribution. Groups were assumed to differ significantly when the probability level was less than 0.05.

RESULTS

Patient data

We recruited a total of 428 transfused SCD patients during the study period. Of these, 217 (51%) were females and among them, 19 (8.8%) had a history of pregnancy. The median age at the time of blood draw was 12 (range, 2-44) years. The patients were transfused with a total of 3,366 (median, 3; range, 2-100) units of blood in 2,463 (median, 3; range, 2-80) transfusion episodes. Twenty six patients (6.1%; 95% CI: 4.0-9.0%) were found to be alloimmunized to RBC antigens; 21 (80.7%) of them having received up to a maximum of 10 blood transfusions. There were 57 patients (13.3%) who had been transfused in childhood and could not recall their exact time when they were first transfused and an age of *3 years* was used in the analysis as their *age of first transfusion*. The number of units of blood transfused was significantly associated with the rate of alloimmunization (p=0.02). There was a trend towards statistical significance between the number of transfusion episodes and the rate of RBC alloimmunization (p=0.08). Other demographic and transfusion characteristics of alloimmunized patients were not significantly different to those in the non-immunized group (Table 1).

TABLE 1: Demographic and transfusion characteristics of SCD patients in Uganda

	Alloimmunized patients	Non-immunized patients	*p*-value
Patients (n, %)	26 (6.1)	402 (93.9)	
Female-to-male ratio	1.8	1.0	NS
Age in years	13 (2-35)	12 (2-44)	NS
History of pregnancy (%)	11.8	8.5	NS
Age of first transfusion < 10years (%)	80.8	89.1	NS
Transfusion episodes	3.5 (2-32)	3 (2-80)	0.08
≤ 10 transfusion episodes (%)	80.7	90.2	NS
Units of blood transfused	5 (2-60)	3 (2-100)	0.02
History suggestive of a 'febrile illness' at the time of transfusion (%)	73.1	70.8	NS
Number of years since the last transfusion	1.0 (0-8)	1.0 (0-8)	NS
Number of years between first and last transfusions	6.0 (0-22)	4.0 (0-41)	NS

Data are presented as median and range unless otherwise stated, NS = *p*-value ≥ 0.1

RBC antibodies

The 26 alloimmunized patients produced a total of 30 RBC alloantibody specificities. This implies that after transfusion with 3,366 RBC units, the alloimmunization rate was 0.9% per RBC unit transfused. Two of the patients possessed panreactive antibodies. Table 2 shows the specificities of the antibodies identified, with 20 (66.7%) belonging to the Rh blood group system. MNS was the next frequent blood group system involved contributing 5 (16.6%) alloantibodies, 4 (80%) of these being of anti-S specificity. Eleven of the alloantibodies (36.7%) presented as multiple antibody combinations. Of the immunized patients with specific antibodies, 19 (79.2%) produced only one antibody while 5 (20.8%) had multiple antibodies.

D genotyping

Seven of the allommunized patients possessed anti-D antibodies and their RHD genotyping revealed the following results: one was D negative; five had partial D (i.e. four patients were of category D^{Va} and one was a probable category D^{IIIb}) and the last had a probable Rh D pseudogene. The D negative individual was a 5-year old girl who had been transfused four times

in the previous two years while the patient with a probable Rh D pseudogene was an 11-year old boy with a history of three blood transfusions since the age of 1 year. Both patients had been typed as D negative by serology. Most likely, they received D positive RBC transfusions despite the local transfusion policy of matching for the D antigen.

TABLE 2: Specificities of RBC alloantibodies identified in 26 SCD patients in Uganda

Blood group system	RBC alloantibody specificity	Number of antibodies (respectively)
Rh	E, D, C, C^w	10, 7, 2, 1
MNS	S, M	4, 1
Kidd	Jk^a	2
Kell	K	1
Duffy	Fy^a	1
Lewis	Le^a	1
N.A.	Panreactive	2

N.A. = not applicable

DISCUSSION

This is the first ever study carried out to determine the frequency and nature of RBC alloimmunization following blood transfusions in Uganda. It also involves one of the largest numbers of SCD patients ever investigated for RBC alloimmunization, in a cross-sectional survey. We observed an RBC alloimmune response rate of 6.1% in 428 SCD patients (or 0.9% per RBC unit transfused) and multiple antibodies were present in 20.8% of immunized patients with specific alloantibodies. Two patients possessed panreactive antibodies 5 years after their last transfusions. However, we could not rule out the presence of post-transfusion autoimmunization because we lacked autologous RBCs and DATs were not done. The number of immunized patients is lower than that reported in most of the literature on RBC alloimmunization in SCD.[4, 5, 8-11, 13, 15] The presumed high phenotypic compatibility between blood donors and SCD patients (who were black Ugandans in both cases) and the low transfusion load may explain the low rate in RBC alloantibody formation. Studies need to be

performed to confirm the phenotypic similarities between blood donors and SCD patients in Uganda. The 6.1% rate of alloantibody formation in Ugandan SCD patients is comparable with the 2.6% alloimmunization reported by Olujohungbe *et al.*[14] in a Jamaican cohort of SCD patients where there was less heterogeneity among donors and patients. It also compares well with the 5.3% frequency of RBC alloimmunization reported in a study by Sarnaik *et al.*[11] in which children with SCD, who were not on a prophylactic transfusion program and had received a low transfusion load, became alloimmunized. The findings in this study show that the rate of RBC alloimmunization was associated with the number of units of blood transfused and the number of transfusion episodes, although the association was not statistically significant in the latter case (the calculated p values were 0.02 and 0.08 respectively). These findings are consistent with previous reports which have shown an association between RBC alloimmunization and increased number of donor exposures.[4, 8 - 10]

The SCD patients mainly received acute simple RBC transfusions and they were not heavily transfused (median number of units of blood transfused = 3) compared to their counterparts in the developed world who may be on chronic transfusion programs. Most patients (71%) and/or their parents described having presented with symptoms of fever, body pains, general weakness or severe anemia at the time of hospital admission and prior to blood transfusion. Unfortunately, the true picture of this 'febrile illness' could not be ascertained from the available medical records but it might have been a sickle cell painful crisis, a delayed hemolytic transfusion reaction (since 11.2% of the patients received repeat transfusions within 2 weeks of a prior transfusion episode), a bacterial infection or even malaria. The role of such an underlying pathophysiology vis-à-vis the rate of alloimmunization needs to be explored in a future prospective study. Recently, inflammation was reported to be associated with increased RBC alloimmunization, in a murine model[27]. Owing to the fact that the patients were not being monitored for alloantibody formation, the rate of RBC alloimmunization may actually be higher than what was observed. This being a cross-sectional study, some RBC antibodies may have been missed because up to 25% of alloantibodies have been reported to disappear within a median 10 months of follow up.[19]

Eighty percent of the detected alloantibodies corresponded to the Rh and MNS system antigens C, D and E; and S respectively (Table 2). Interestingly, 7 (23.3%) of the patients formed anti-D alloantibodies notwithstanding the local clinical transfusion practice in which ABO/D group

compatible blood is transfused. Moreover, 5 (71%) of the patients who produced anti-D were females within the age range of 5 – 19 years and with no history of pregnancy. Since some of these patients had been typed as D positive by serology, we decided to investigate the molecular bases underlying these observations. Using an *RHD*-specific multiplex PCR, D genotyping of the 7 SCD patients who were D-alloimmunized revealed that one of them was D negative, five had partial D (D^{Va} in four patients and a probable D^{IIIb} in the other), and the last one had a probable Rh D pseudogene (D negative by serology; all amplified exons present). The patient who was probably D^{IIIb} had all the six RHD exons (3, 4, 5, 6, 7 and 9) tested and had been found to be D positive by serology. We could not proceed to perform DNA sequencing for confirmation of the probable RhD pseudogene or the D^{IIIb} category because there was no more DNA available. These findings underscore the need to use monoclonal anti-D reagents that are capable of detecting D variants among blood donors and recipients and to improve the standards of immunohematological testing in Uganda. The antibodies encountered in other series[3, 4, 5, 10, 28] have most commonly been of C, E or K specificities. To prevent alloimmunization in SCD patients in the United States and Europe, the standard practice is to perform antigen matching for C, E, and K antigens for patients without prior alloantibody formation.[29] Our findings indicate that anti-K is rare (3.3%) while anti-S is more common (13.3%) among alloimmunized SCD patients in this study. This is presumably because of a difference in the distribution of Kell and MNS phenotypes in Caucasian and Black populations. Accordingly, anti-S should be borne in mind in case a program of *limited* phenotype matching (i.e. for C, E and S antigens) to improve the care of already alloimmunized SCD patients in Uganda is to be considered in future.

The effects of RBC alloimmunization in SCD patients may be examined in the context of the policy on laboratory and clinical transfusion practice in Uganda. This study has revealed the presence of clinically significant IgG alloantibodies in plasma of transfused SCD patients. Transfusion-acquired antibodies have been implicated in immediate and delayed transfusion reactions;[2, 10, 16] some patients with multiple antibodies are difficult to cross-match and to transfuse;[8, 21] others develop autoantibodies in addition to being alloimmunized;[28, 30] and five nulliparous females in the present study were alloimmunized to the D antigen. The current transfusion practice in Uganda does not involve the detection or monitoring of alloantibody formation and the clinical consequences thereof. Besides ABO/D grouping and a saline cross-match at room temperature, no other compatibility testing is performed. Therefore, we

recommend a change in the policy of the Uganda Blood Transfusion Service to include laboratory and clinical guidelines on the prevention and management of immunological complications of allogeneic blood transfusions, including RBC alloimmunization in SCD patients.

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