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Red Blood Cell Alloimmunization among Sudanese Homozygous Sickle Cell Disease Patients

Mohammed Abbas[1,*], Ahmed Bolad[2], Nasreldin Jiefri[3], Adil Mergani[4]

[1]College of Applied Medical Sciences, Medical Laboratory Department, Salman bin Abdulaziz University, Riyadh-Al Kharj, 11942, Saudi Arabia (KSA)
[2]Department of Microbiology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan
[3]Hematology department, Faculty of Medicine , Al Neelain University , Khartoum, Sudan
[4]Department of Molecular Biology and Biotechnology, College of Applied Medical Sciences, Medical Laboratory Department, Taif University, –Tariba, Taif, Saudi Arabia (KSA)

Abstract Background: Transfusion of RBCs is a major therapeutic option for anemia in homozygous SCD. Numerous studies conducted in the world to determine the frequency of alloimmunization in SCD patients, but there are no studies conducted in Sudan in open literature, to our knowledge. The objective of this study was to assess the incidence of RBC alloimmunization in SCD HbSS patients in Sudan. Methods: This study was conducted in Omdurman between August 2011 - Aril 2012, in 100 SCD HbSS patients of age between 6 month –17 years, who had received at least 2 transfusions with units of ABO and D matched RBCs, the alloimmunization was determined. Results: The mean No of blood units transfused per patient was 6.73 (SD, 4.461). 55% of the patients had been transfused from non relative donors, while 45% from relative donors. Only four patients was found to have antibodies 4% were detected among the 100 SCD (HbSS) patients who received transfusion. Conclusions: Although limited precompatibility testing is done in blood banking services in Sudanese hospital, low incidence of red cell alloimmunization (4%) was observed in this study. We recommend that to improve the blood bank services and polices to overcome the risk of alloimmunization.

Keywords SCD, Transfusion, Alloimmunization

1. Introduction

Sickle cell disease (SCD) is an inherited disorder of hemoglobin (Hb), it is the most prevalent genetic disease in African region[1] with high mortality rate at age one to five years.[2] Since description of the first case of SCD in Sudan at 1926.[3] There was and still increasing research activities in this field. The highest prevalence of SCD in Sudanese is among the population from the western Sudan.[4-6]

SCD is caused by an autosomal recessive inherited of an abnormal beta globin gene (sickle cell gene) which leads to substitution of thymine by adenine in glutamic acid which in turn results in the substitution of valine to glutamic acid at the sixth position on the beta globin chain. Hemoglobin S (HbS), is the defective Hb that produced as a result of this defect, is a tetramer (alpha2/beta2) that is poorly soluble and polymerizes when deoxygenated.[7] The two characteristic features in the pathophysiology of SCD is the chronic hemolytic anemia and the vaso-occlusion.[8]

Transfusion of red blood cells is a major therapeutic option in SCD. It improve the oxygen-carrying capacity by increasing the total Hb level, decrease blood viscosity and increasing oxygen saturation by diluting the concentration of HbS; and suppressing endogenous production of sickle RBCs by increasing tissue oxygenation[9-11].

The repeated or chronic RBC transfusions and the exposure to different red cells antigen are often complicated by RBC alloimmunization, which results in difficulties in the selection of compatible blood, acute or delayed transfusion reactions.[12] A considerable number of studies have dealt with Alloimmunization in SCD, the range of RBCs alloimmunization has been reported to be in the range of 2.6% to 76%.[13-29,50].

The precompatibility testing procedures in the majority of the Sudanese hospital is limited to perform only ABO Rh grouping, and cross-matching by using saline and anti human globulin techniques by tube method. Although some major hospitals start to use the column technique in across-matching procedures, but they still use limited tests. There are no antibody screening , limited phenotyping, or even further antibody identification were done as a routine work far as we know.

Despite of the numerous studies conducted in the world to determine the frequency of red cells alloimmunization in SCD patients[13-29,50], unfortunately there are no enough

* Corresponding author:
myamya88@hotmail.com (Mohammed Abbas)
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studies conducted in Sudan in open literature, to our knowledge. The objective of this study was to assess the incidence of RBC alloimmunization in SCD patients in Sudan.

2. Material and Method

After appropriate ethical approval prior the commencement of the study, a cross-sectional study was carried out, random samples were analyzed. 5 ml of venous blood in K2 ethylenediaminetetraacetic acid (EDTA) for each sample. Questionnaires were used to collect demographic and clinical data include sex, age, tribe, number and indication for transfusion, transfusion reaction, and malarial attack . A total of hundred (100) homozygous sickle cell disease (HbSS) patients of age between 6 month –17 years, both males (57%) and females (43%) belonging to different tribes who had received at least 2 transfusions with units of ABO and D matched RBCs were selected from Albuluk pediatric teaching hospital and Omdurman pediatric hospital between August 2011 and Aril 2012. The number of blood transfusion ranged from 2 to 20 units per patient, the total number of transfusion to all patients was 673 units.

2.1. Laboratory Investigation

After ABO and Rh blood grouping by the standard tube method, the following tests were done for every sample:

Antibody screening. The collected plasma were tested for the presence of alloantibodies using a 3-cell panel with homozygous expression of the antigens (Panoscreen I,II and III, Immucor, Inc, USA) by using saline phase and indirect human antiglobulin test (indirect Coombs) in low-ionic-strength solution (LISS) by the tube method.

Antibody identification. Antibody specificity was determined with a standard panel of red cells reacting to known antigens using the same technique in the positive antibody screening (Panocell -10, Immucor, Inc, USA).

Patients were considered to be alloimmunized if antibodies to one or more RBC antigens were identified.

2.2. Data Analysis

Statistical software packages (Excel 5.0, Microsoft, Redmond, WA; and Statistical Package for the Social Sciences 20.0, SPSS, Inc., Chicago, IL) were used for data management and analysis, respectively.

3. Results

A total of 100 patients with SCD (HbSS) formed the study population. Fifty seven patients were males (57%), and forty three were female (43%) of age between 6 month – 17 years. (67%) of patients ages fall between 2 and 8 years, (25%) between 9 and 17 years and the lower proportion of patients aged less than two years (8%). Over (97%) of the patients involved in this study belong to tribes from the West of Sudan. Miserria, Marareet, Rizegat and Bargo constituted the largest proportions of the tribes, (28%, 22%, 12% and 8% respectively). Other tribes represents low percentage include: Tama, Habbania, Jawamaa, Zagawa, Masalma, Slihab, Bnehalba and Kwahla. (59%) of the patients parents are close relatives, (26%) are tribal, (5%) are far relation, while (10%) are not relatives. Only (2%) of the patients has had a history of malarial attack.

Figure 1 shows the rank order of blood group among subjects: O Rh + (n = 45 patients), A Rh + (n = 22), B Rh + (n = 20), AB Rh + (n = 8) , B Rh – (n = 2) ,A Rh – (n = 2) ,O Rh – (n = 1).

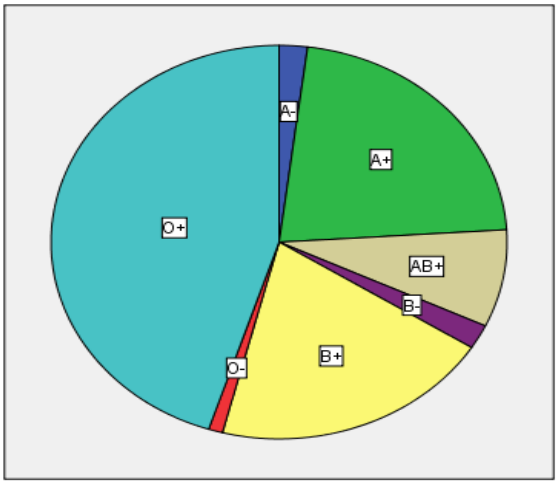

Figure 1. The frequency of ABO RH group among patients

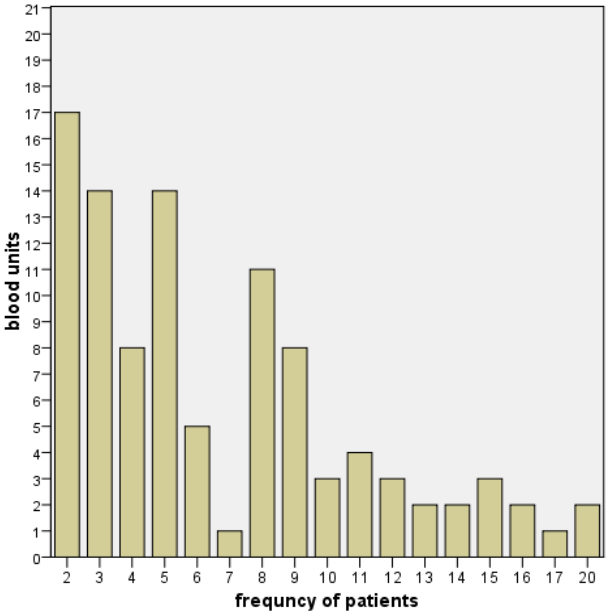

Figure 2. The frequency of blood units taken by the patients

The patients were transfused with a total of 673 (SD, 4.461; median, 5; mean, 6.73; maximum, 20; minimum, 2) units of blood. Figure 2 shows the frequency of blood units taken by the patients. (55%) of the patients were transfused from non related donor, while the residue (45%) from relative donor. Only one patient (1%) showed delayed

transfusion reaction and another one showed immediate reaction.

Four antibodies (4%) were detected among the 100 SCD (HbSS) patients who received transfusion. Anti-K was detected in two patients, while anti-C and anti-E were detected in another patients, Table 1 shows the profile of the patients who developed alloantibodies in this study. Three of four patients (75%) showed no relation to donors. The patients who developed the alloantibodies received the higher rate of transfusion (between 15-20 units). There were no red cell antigens autoantibodies detected in patients samples.

4. Discussion

The alloimmunization is considered one of the major blood transfusion complications in SCD patients who received regular transfusion therapy, unfortunately, there are no published studies to determine the frequency of alloimmunization in SCD Sudanese patients as far as we know. In this study we try to establish the incidence of the alloimmunization in this category in Sudan.

The sickle cell anaemia (SCA) is the one of the major types of anaemia especially in Kordofan and Darfur states[4], western Sudan. Over (97%) of the patients involved in this study belong to tribes from that area, Elderdery, A[30] also noticed the high frequency of homozygous HbSS in his study among western Sudan tribes. Misseria which is a sub group of Baggara tribe, showed the highest percentage of the entire study group of patients in this study (28%). This tribe showed the prevalence of SCD to be (30%), (16%) among immigrants from the province of Blue Nile[31].

Blood group O (46%) was predominant among patients blood groups, this finding in agreement with three previous studies performed among Sudanese (52.7%) ,(51.5%) and (43.7%)[32-34], respectively. The result also consistent with other studies performed in African countries, shown an increase in group O percentage : Nairobi- Kenya (69%)[35], Nigeria (48.9%)[36]. The predominance of group O also observed in Western countries, in UK blood group O constitute (46.7%)[37], (47%) and (46%) of Caucasians and blacks in USA were group O respectively[38]. The increase percentage of group O in this study may be related to the low incidence of malarial attack (2%), which in agreement with some findings suggested the diverse relations between group O and different types of malaria[39-42]. Rowe JA et al; observed that group O was associated with a (66%) reduction in the odds of developing severe malaria compared with the non-O blood groups[43]. Furthermore, there is some convincing evidence to suggest that patients with SCD are protected from malaria[44-48]. The low prevalence of malaria observed in this study is matched with that reported by Makani J, et al.[49] the prevalence of malaria parasitemia was (0.72%) of 1808 SCA patients at the outpatient clinics and (3.04%) of 497 SCA patients during hospitalization.

Table 1. Profile of homozygous SCD patients who developed alloantibodies

Patient No	gender	age	tribe	Parent relation	Blood group	Blood units	Patient donor relation	alloantibody detected	Transfusion reaction	Indication for transfusion
1	M	10	Bargo	close	O +	15	No	Anti-C	No	Painful crisis
2	F	7	Habbania	Not related	O+	20	No	Anti-K	No	Anemia Hb<7g/dl
3	F	3	Marareet	close	O+	17	Yes	Anti-E	Late jaundice	Joint pain+ anemia Hb<8g/dl
4	M	4	Jwamaa	tribal	A+	16	No	Anti-K	No	anemia Hb<8g/dl

Table 2. review of alloimmunization in patients with homozygous SCD and the prevalence of anti-K, anti-C, and anti-E alloantibodies

Region	First author (year)	Country	Rate of alloimmunization	Anti-K	Anti-C	Anti-E
North and South America	Wendell F. Rosse (1990)[17]	USA	18.6%	28.1%	30.2%	42.3%
	Vichinsky EP (1990)[51]	USA	30%	26%	16%	24%
	Cox JV (1988)[19]	Brazil	9.9%	9.7%	18.5%	33.1%
	Olujohungbe A (2001)[22]	Jamaica	2.6%	NR	NR	0.5%
Europe	Olujohungbe A (2001)[22]	UK	76%	13.5%	13.5%	13.5%
	Zalpuri, S (2011)[29]	Netherlands	6.5%	21.7%	2.9%	39.1%
Asia	Bashawri LA (2007)[23]	KSA	13.7%	10.4%	1.4%*	18.8%
Africa	Natukunda B (2010)[27]	Uganda	6.1%	3.3%	6.7%	33.3%
	Batina-Agasa S (2010)[51]	DRC	10%	NR	NR	NR
	Rabab Aly (2012)[50]	Egypt	21.4%	7.1%	4.8%	4.8%
	present study (2013)	Sudan	4%	2%	1%	1%

*in combination with other alloantibodies
NR: not reported

The range of RBCs alloimmunization in a considerable number of previous studies has been reported to be in the range of 2.6% to 76%.[13-29,and 50]. Table 2 illustrate the frequency of RBCs alloimmunization in a number of studies conducted in different countries and the frequency of anti-K, anti-C and anti-E. This wide variation could be attributed to the transfusion load, closer matching of donor to recipient and the heterogeneity of the population in the community which may lead to increase the frequency of uncommon blood group antigens. The results of this study demonstrated that alloantibodies were developed in 4% of the patients, a frequency consider within the lower limit determined in literatures. The 4% rate of alloantibody formation in Sudanese SCD patients is incomparable with the 6.1% alloimmunization in neighboring regional study among Ugandan SCD patients reported by Natukunda B and colleagues[27] where there was less heterogeneity among donors and patients. Other studies carried out in African countries also reported low alloimmunization rate: (10%) in Democratic Republic of Congo (DRC)[51] and (7.75%) in Tunisia[52]. The low frequency of alloimmunization (2.6%) in Jamaica was anticipated by the author to the conservative use of transfusion there (range 1-22)[22], this finding matched with the range of blood unit taken by the patients in this study (2-20). The low frequency of alloimmunization in the current study may be attributed to the homogenicity of ethnic population since all donor although (55%) of them were not related to the recipient but there were Sudanese. The relatively low number of blood units taken (mean, 6.73) also may explain the low frequency. Young age of participants in this study consider an important factor in minimizing the alloantibodies frequency this finding in agreement with several studies[17,20,52,and 61]. The high rate of alloimmunization (21.4%) reported by Rabab Aly in 42 Egyptian SCA patients with mean age 12 year[50] is not matched neither with the result of alloimmunization in this study, nor with the results in other African countries[27,51and 52], the studied patients received from 4 to 12 units of blood per year. (11.3%) of a total 501 transfusion dependent Egyptian thalassemic patients of age between two and twenty six years were develop red cells alloantibodies[53]. The differences between the sample size in the previous studies together with the RBCs antigenic phenotypes variations between the patients and donors even they were from the same nationality especially they received approximately the same rate of blood transfusion per year (2 12 units/year), may explain the distinction in the rate of red cell alloimmunization among Egyptian patients receiving chronic transfusion. The rate of alloimmunization is higher in USA and UK compared to Uganda, DRC, and the present study. This may be explained by the assumed high phenotypic compatibility between blood donors and SCD patients in African countries, the most donors in the United States of America are Caucasian and SCA recipients are almost exclusively of African ancestry; this has been reported to be a cause for a high rate alloimmunization in these patients[54], Furthermore the greater red cell alloimmunization reported among UK SCD patients reflects the racial disparity between donor and recipient populations in the UK as well as greater use of transfusions[22].

The alloantibodies to the Kell system antigen K as well as to the Rhesus system antigens C and E, are comprise the percentage of the RBCs alloimmunization in this study, this finding in is in accordance with others[12,17,20, and 50]. The low frequency of K antigen among Sudanese (5.6%)[55] , together with unrelated donor to recipient may explain the present of anti-K alloantibody in two patients in this study. The frequency of antigen E and C is also relatively lower in percentage in comparison to antigen c, e and D among Sudanese[56]. The rhesus antigens are characterized by high immunogenicity[57] which intern lead to the formation of alloantibodies when transfused to the recipient lack such antigens. Anti-C anti-E appeared to be frequent alloantibodies in many studies[17,19,20,22,,27,29 and 51]. In this study the formation of Anti-C anti-E also may be attributed to the decrease their frequency in Sudanese population and to the unrelated donor. The phenotype matched donor RBCs viewed benefits to chronically transfused SCD, in one study the rate of alloimmunization dropped from 3% to 0.5%[21]

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 this finding in agreement with many studies observed that the RBCs alloimmunization is more likely in patients receiving multiple transfusion[13-20,27,50,54,58-60]. The patients who developed the alloantibodies received the higher rate of transfusion (between 15 -20 units) , their age range between 3 to10 years (Table 1) .

There were no red cell antigens autoantibodies detected in patients samples. The increase frequency of alloimmunization in females compared with male was observed in number of studies[13,50 and 54] the result in the current study not in harmony with that finding, there was no observable gender based differences in formation of alloantibodies.

5. Conclusions

The present study is the first study to determine the incidence of alloimmunization among homozygous SCD Sudanese patients. Although the limited tests were performed as a routine precompatibility testing in blood banks in Sudan hospitals, but fortunately low incidence of alloimmunization was detected (4%) which can be compared with other studies with low incidence of alloimmunization [22,27, and 29]. We recommend that to improve the blood bank services and polices in the way of RBCs compatibility tests, limited phenotype matching in Kell and Rhesus blood group systems specially in K, C, and E antigens, and improve the laboratory techniques to involve the alloantibody detection and identification. After making these improvement the alloantibodies formation in chronically transfused patients can easily be prevented.

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Conflict of Interest

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

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