

# Red Blood Cell Alloimmunization in Sickle Cell Disease Patients in Jeddah, Saudi Arabia: A Pilot Study

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## Abstract

The treatment of patients with sickle cell disease frequently requires transfusion of red blood cells. Complications due to alloimmunization of red blood cells antigen remain a major risk as a post transfusion effect. The objective of this study is to determine the frequency of red cell alloimmunization in Jeddah, Saudi Arabia. A retrospective cross-section study of sickle cell disease patients at King Abdulaziz University Hospital between 2012-2013 was performed. Demographic characteristics and transfusion history was recorded. Blood samples were analysed for alloimmunization using immunohematological technique. A total of 234 sickle cell patients were analysed, of which 30 (12.8%) showed alloantibodies. A total of 43 alloantibodies were found out of which 28 belonged to Rh group, eight belonged to Kell while three belonged to MNS group. Demographic and transfusion characteristics were analysed between alloimmunized and non-alloimmunized sickle cell disease patients. The rate of alloimmunization in Jeddah, Saudi Arabia was 12.8%. There was significant difference observed between alloantibodies detection between transfused patients compared to non-transfused patients. The consequences of red blood cell alloimmunization are highly significant and therefore immune haematological testing is highly recommended.

## Keywords

Alloimmunization; Sickle cell disease; Red blood cell; Transfusion

## Introduction

With the advancement in availability of erythrocytapheresis and improved methods to treat with iron overload, transfusion therapy is

increasingly used for the treatment of sickle cell disease (SCD). Red blood cell alloantibody formation remains a major complication for patients with SCD, making blood transfusion a medical challenge. With the increased use of transfusion techniques for the management

of SCD patients, the alloimmunization issue becomes imperative. The incidence of alloimmunization in SCD patients ranges from 7% to 47% depending on age, ethnicity, gender, RBC exposure and antigen mismatch<sup>[1-5]</sup>. Out of those who possess alloantibodies 4-11% have delayed hemolytic transfusion reactions (DHTR)<sup>[6]</sup>.

In the United States multiple red blood cell RBC alloimmunization antigens were detected in more than 50% of alloimmunized subjects<sup>[7]</sup>. Similar reports have been documented from Brazil with 50% of the patients with SCD (56 out of 108) exhibiting alloimmunization<sup>[8]</sup>. However in Uganda, only 6.1% possessed alloantibodies<sup>[9]</sup>. Studies in Eastern Saudi Arabia documented alloantibodies in 13.7%<sup>[10]</sup> while another similar study revealed an alloimmunization rate of 34.2%<sup>[11]</sup>.

The Rhesus (Rh) system comprising of two homologous genes, RHD and RHCE which encode D antigen and CE antigens in various combinations includes 50 different serological specificities<sup>[12]</sup>. Along with Rh and K antigen, minor antigens in Kidd, Duffy and MNS are found at lower rates<sup>[13]</sup>. One approach to minimize alloimmunization is to transfuse phenotypic matched RBC to the SCD patients.

Indications for transfusion include acute aplastic crisis, acute splenic or hepatic sequestration, symptomatic anemia, stroke treatment and prevention, acute chest syndrome treatment and prevention, and before preparation for major surgery<sup>[14,15]</sup>. Randomized controlled trials have demonstrated that prophylactic red cell transfusions significantly decrease the frequency of stroke events in at-risk paediatric patients with SCD<sup>[16]</sup>.

The objective of this study was to establish the frequency, and characterize the nature of, RBC alloimmunization in SCD patients of Saudi Arabia.

## Materials and Methods

### Patients

This is a retrospective cross-sectional study carried out at King Abdulaziz University Hospital between 2012 and 2013 comprised of patients coming to the hematology outpatient clinic. Informed consent was obtained from the patients or their parents/guardians. All the SCD patients were eligible for this study. However, those SCD patients having other complications were excluded from the study. A total

of 234 SCD patients' medical records were reviewed. Patients' demographics were analysed and classified according to age, sex, gender, ethnicity, blood group, transfusion history, splenectomy, antibody formation and mortality.

### Laboratory Investigation

Blood was drawn from the patients and plasma samples were screened for the presence of RBC antibodies with polyspecific anti human globulin. Gel technique was used for detection and identification of antibodies. Patients' RBCs were serologically phenotyped before transfusion for ABO; Rh (D, C, c, E, e); Kell (K); Duffy (Fy<sup>a</sup>, Fy<sup>b</sup>); Kidd (Jk<sup>a</sup>, Jk<sup>b</sup>); Lewis (Le<sup>a</sup>, Le<sup>b</sup>); MNS(M,N,S, s) and PI.

### Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20. (IBM Corp., Armonk, NY USA). Significance and frequency tests were performed on alloimmunized and non alloimmunized patients regarding all the parameters. Groups were assumed to be significant when probability level was less than 0.05.

## Results

Study period was 2012 to 2013, and 234 sickle cell patients were screened for the presence of alloantibodies. 30 (12.8%) out of 234 showed alloimmunobodies. Out of the 30 patients showing alloantibodies, 28 were Rh+ and 2 were Rh-. The demographic and transfusion characteristic of SCD patients are given in Table 1. The specification of alloantibodies identified in the 30 SCD patients is given in Table 2.

## Discussion

Despite improved patient management with hydroxyurea, transfusion technique of management is associated with a considerable reduction in morbidity and mortality of SCD patients. Conversely, transfusion brings serious complications due to erythrocytes alloimmunization. This study reveals the frequency and nature of alloimmunization in post transfused SCD patients in Jeddah Saudi Arabia. It is the first study in Western Saudi Arabia accounting the presence of, quantity, and type of antibodies in SCD patients. Out of the total 234 SCD patients 131 were male and 103 were female. The mean age was 24 (range, 7-54). The patients were transfused between

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**Table 1.** Demographic and transfusion characteristic of SCD patients in Saudi Arabia.

Demographic	Alloimmunized Patients		Non-Alloimmunized Patients		p-Value*
	No of Patients	%	No of Patients	%	NS
No of Patients	30	12.80%	204	87.20%	NS
Female/Male	14/16	46.70%/53.30%	89/115	43.60%/56.40%	NS
Saudi/Non Saudi	6/24	20.00%/80.00%	98/106	48.00%/52.00%	NS
Age in Years	10-42		7-54		
Transfusion Episode	20	66.60%	91	44.60%	0.026 <sup>†</sup>
3-4 weeks	3	10.00%	16	7.80%	
5-7 weeks	6	20.00%	17	8.30%	
≥ 8 weeks	11	36.60%	58	28.40%	
No of Transfusions	10	33.30%	113	55.40%	
Deceased	1	3.30%	8	3.90%	NS
Rh Positive/Rh Negative	28/2	93.00%/7.00%	189/15	92.60%/7.40%	NS
ABO Blood Group					
A	15	50.00%	62	30.40%	NS
B	2	6.70%	24	11.80%	NS
O	12	40.00%	108	52.90%	NS
AB	1	3.30%	10	4.90%	NS
Splenectomy					NS
Yes	4	13.30%	15	7.40%	
No	26	86.70%	189	92.60%	

\*P values comparing the continuous variables were obtained by independent-samples t-test, IBM SPSS Statistics for Windows, Version 20.

<sup>†</sup>Significance p < 0.05; NS= Non Significant

**Table 2.** Specificities of 43 RBC alloantibodies identified in 30 patients in Saudi Arabia.

Blood Group System	RBC Alloantibody (Percentage of Alloantibodies Identified)	RBC Alloantibody Specificity	Number of Antibodies (Respectively)
Rh	65	E, D, C	16,2,10
MNS	18.6	S M	2,1
Kell	7	K	8
Duffy	4.6	Fy	2
Lewis	2.3	Le <sup>a</sup>	1
Luth	2.3	Lu <sup>a</sup>	1

3-4 weeks, 5-6 weeks or after 8 weeks according to the requirement. Thirty (12.8%) patients were found to be alloimmunized to RBC antigens. The frequency of alloimmunization in different populations ranges considerably depending on various factors. In the United Kingdom, United States, Brazil and Kuwait the documented alloimmunization rates in patients with SCD was between 18-76%<sup>[3,8,17-19]</sup>, while in Africa it showed 6.1%<sup>[9,20]</sup>. In a Michigan study the frequency rate of RBC alloimmunization was reported to be 5.3% unlike other United States studies. Studies carried out in Eastern Saudi Arabia on SCD patients documented alloantibodies in 13.7% (48 out of 350) while another similar study revealed an alloimmunization rate of 34.2% (38 out of 111)<sup>[10]</sup>. The results of the first study are similar to this study. However the older study shows a higher rate of alloimmunization, which could be due to the smaller sample size.

The varied range of alloimmunization is due to racial and ethnic differences between donors and recipients of transfused RBC. The greater the genotypic antigenic mismatch, the higher the alloimmunization is instigated. In Saudi Arabia there is a mixed population and there is a strong possibility of mismatches.

Age has been correlated with the risk of alloimmunization by Rosse *et al.*<sup>[20]</sup>. The study describes a higher frequency in children who receive transfusion after the age of 10. However in the present study, all the patients showing alloimmunization were above 10 years. There was no significant difference between the age of patients who developed antibodies and those who did not develop alloantibodies (Table 1). Similar results were documented by Dias Zanette *et al.*<sup>[8]</sup> in 2010 showing that the age of the first transfusion had

no significant difference between alloimmunized and non alloimmunized patients. There were 16 males and 14 females in the alloimmunized group, while 115 males and 89 females were in the non alloimmunized group. There was no significant difference between the two groups and also no difference within the groups. All the other demographic data are given in Table 1. Those patients who got transfusion showed significantly higher antibodies compared to those not transfused. All other parameters showed no significant differences.

The total number of immune antibodies found in this study were 43, out of which Rh and Kell group showed maximum antigen, the rest belong to MNS, Duffy, Lewis and Luth antigens. Table 2 shows the specificity of antibodies identified, with the Rh blood group system having 28 (65%) antibodies, Kell being the next frequent blood group involved with 8 (18.6%) alloantibodies, while MNS was the next frequent blood group contributing 3 (7%) alloantibodies. Finally Duffy, Lewis and Luth showed 2 (4.6%), 1 (2.3%) and 1 (2.3%) alloantibodies, respectively.

In the alloimmunized patients, two patients were Rh D negative blood type. One patient was detected having alloantibody C and D of Rh blood group system while a second patient showed only alloanti-D. As a referral hospital we do receive patients who have a previous history of blood transfusion in another hospital and subsequently developed a complication of antibodies to red cells. Patients also tend to move between hospitals to receive blood. In all hospitals antibody screening is not done. We recommend that national guidelines, policies and training of staff should be encouraged to improve blood transfusion services and to minimize red cell antibodies in transfusion dependent patients.

The National Institutes of Health recommends that a RBC phenotype (ABO, Rh, Kell, Duffy, Kidd, Lewis, Lutheran, P, and MNS at a minimum) be obtained on all patients with SCD older than 6 months<sup>[21]</sup>. To prevent alloimmunization in SCD patients, the standard practice is to perform antigen matching for C, E and K<sup>[2]</sup>. In the present study, we have maximum alloimmunity from the antigen recommended by National Institutes of Health. Therefore the similar strategy can be applied with additional S antigen testing of MNS system. The only limitation of this study is the small sample size. A similar study with increased sample size is our subsequent targeted study.

## Conclusion

To improve and manage the consequences of alloantibodies in sickle cell patients, performing an antibody screen with subsequent antibody identification is obligatory for all transfused patients at King Abdulaziz University Hospital and a few other hospitals in Saudi Arabia. The testing should be done for RH and Kell blood group system as mandatory, while MNS, Duffy, Lewis, Kidd and Luth blood group system is advisable. Transfusion medicine specialists can play an important role by making this a routine practice for the welfare of SCD patients. Transfusion problems can be largely excluded by the execution of preventive measures. There is a large variation in transfusion management among paediatric hematologists and related practices<sup>[22]</sup>. The development of comprehensive guidelines and protocols for transfusion requirements in SCD must be developed and practiced globally. There is an explicit need for prospective clinical trials addressing phenotype matched blood transfusion in SCD patients.

## Conflict of Interest

The authors have no conflict of interest.

## Disclosure

None of the authors received any type of commercial support either in forms of compensation or financial for this study. They have no financial interest in any of the products or devices, or drugs mentioned in this article.

## Ethical Approval

Obtained.

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## تكوين أجسام مناعية عند مرضى الأنيميا المنجلية الذين يتلقون نقل الدم بجدة، المملكة العربية السعودية: دراسة تجريبية.

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**المستخلص.** كثيراً ما يحتاج مرضى الأنيميا المنجلية إلى نقل دم مما ينتج عنه تكون أجسام مناعية مضادة للكريات الحمراء. أجريت هذه الدراسة بأثر رجعي على شريحة عريضة من مرضى الأنيميا المنجلية بمستشفى جامعة الملك عبد العزيز بجدة، ما بين ٢٠٠٢ إلى ٢٠١٣م. تم تسجيل التوزيع الديموغرافي وتاريخ نقل الدم للمرضى. تم إجراء فحص دم للكشف عن الأجسام المضادة باستخدام الإختبارات المناعية الحديثة. أجريت الإختبارات على مجموعة ٢٣٤ مريض تبين وجود أجسام مناعية مضادة في ٣٠ منهم (١٢,٨%). كان مجموع الأجسام المضادة المكتشفة ٤٣ نوعاً منها ٢٨ من مضادات الفصيلة Kell بينما كان هناك ٣ حالات تنتمي إلى فئة MNS. تم تحليل التركيبة الديموغرافية وتاريخ نقل الدم بين المرضى الذين ظهر لديهم تلك الأجسام المضادة والمرضى الذين لم يظهر لديهم أجسام مضادة. بلغ معدل تكون الأجسام المناعية في جدة ( المملكة العربية السعودية ) ١٢,٨% وكان الاختلاف كبيراً بين المرضى الذين تلقوا نقل الدم بالمقارنة مع المرضى الذين لم يتلقوا نقل الدم. إن عواقب ظهور هذه الأجسام المضادة كثيرة ومؤثرة مما يستدعي إجراء فحص التركيبة المناعية للمرضى.