Journal of King Saud University – Science 34 (2022) 102161

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Associations between alloantibodies and multiple red blood cell transfusions in patients with sickle cell anemia

Fahad M. Aldakheel [a,*], Bader H. Alali [a], Shatha A. Alduraywish [b], Ayesha Mateen [a], Rabbani Syed [c]

[a] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh, 11433, Saudi Arabia
[b] Department of Family and Community Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[c] Department of Pharmaceutics, College of Pharmacy, PO Box 2457, King Saud University, Riyadh-11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 27 January 2022
Revised 1 June 2022
Accepted 6 June 2022
Available online 9 June 2022

Keywords:
Alloantibodies
Alloimmunization
Blood Transfusion
Phenotype
Sickle Cell Anemia

ABSTRACT

Alloantibodies are popularly transfusion-related and can be formed against many blood components including the red blood cells, white blood cells, and platelets. The formation of alloantibodies which is a common a sequence for repeated red blood cell transfusion has been reported and are clinically crucial in pregnancies, and to identify risk of future transfusion-related adverse hemolytic reactions. The present study was focused at studying the background clinical factors around red blood cell (RBC) transfusion with respect to alloimmunization, effect of Rh-Kell antigen matching units and its impact on sickle cell anemia (SCA) patients to decrease the incidence of alloantibody development. Methodology – Our retrospective study involved data analysis from SCA patients including pregnant women who had undergone antibody screening from Riyadh, Saudi Arabia. Data from the year 2010 up until 2020 was collected from the King Saud Medical City, the regional laboratory and central blood bank in Riyadh and included a total of 246 patients who matched our criteria. Data from indirect antiglobulin test (IAT) done on each patient at two separate time periods was analysed by appropriate statistical tools in SPSS® software platform. Results – A total of 246 SCA patients with a mean age of 11.9 ± 6.7 years were identified to have obtained blood donation in the study period, and 52.4% transfusion recipients were recorded to have undergone phenotype match: Rh-K. Our study also found 17.9% of the alloimmunized patients to have a record of blood transfusions before alloimmunization. Anti-E (40.9%), anti-Kell (34.1%), anti-D (4.5%) were the most popular specificities of alloantibodies defined. No association was found between gender and production of antibodies, and the backward step binary logistic regression analysis predicted recipients of blood transfusions (>7 units) was 3.66 times more exposed to risk of developing alloantibodies (1.23 – 10.85, 95% CI). While among those in the matching phenotype (Rh-K) group was less likely [OR = 0.012 (0.001–0.11, 95% CI)] to develop alloantibodies. The current study showed that advancing age, and multiple transfusion episodes to increase the risk of developing alloantibodies following blood transfusion among SCA patients.

1. Introduction

Sickle cell Anemia (SCA) involves blood transfusion as a major therapeutic intervention to improve oxygen levels in the blood and reduce risk of vaso-occlusion. Studies have shown long-term transfusion to help in maintaining the HbS% to between 30 and 40% with high hemoglobin (Hb) levels, and significant reduced risk for hyper viscosity. However, blood transfusion has number of risks including iron overload, infection, and alloimmunization (Howard, 2016). Studies have compared the efficacy of conservative (aiming for Hb 10 g/dL), and aggressive (aiming for Hb of 10 g/dL and HbS < 30%) preoperative blood transfusion among SCA patients and found alloimmunization to the most common complication among the aggressive transfusion group (Vichinsky et al., 1995). Most of the study reports on alloimmunization and its risks have been done on clinical patients who have undergone chronic blood transfusion for conditions including hematologic malignancies, hemoglobinopathies, and organ transplant

* Corresponding author.
E-mail address: faldakheel@ksu.edu.sa (F.M. Aldakheel).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.102161
1018-3647/

recipients, among whom the risk of alloimmunization has been reported to be up to 60% (Schonewille et al., 1999; Rosse et al., 1990; Ramsey et al., 1989; Redman et al., 1996; Schonewille et al., 2006).

Transfusion of red blood cells (RBCs) leads to formation of the RBC alloantibodies which are clinically significant to be assayed for to predict risk of hemolytic adverse events in future transfusions and the RBC antigens can be proteins as well as carbohydrates (Reid and Mohandas, 2004). Further, animal studies on immunogenicity indicate low density antigens including KEL or weak RhD exhibit low levels of immunogenicity (Arthur et al. 2017). Study reports on impact of immunosuppressant on alloimmunization have found the incidence to be lower among patients using immunosuppressant [adjusted relative rate = 0.55 (0.34–0.91), 95% CI] (Zalpuri et al., 2014). Apart from incidence of alloimmunization, many studies have also assessed the type of alloantibody and its association if any with frequency of transfusion, donor ethnicity, and gender. One such study by Bhuva and Vachhani (2017) identified alloimmunization frequency to be 3% among patients and the categories to be Kell blood group (55.55%), and Rhesus system (44.44%). Further, the Kell antibodies included anti-K and anti-Kp, while Rhesus alloantibodies included anti-D, and anti-E. Another recent publication involving 200 multi-transfused Egyptian beta-thalassemia patients detected alloantibodies in 18% of patients, and the dominant ones were Kell (33%), and Rh (24.4%) (El-Beshlawy et al., 2020).

Alloimmunization though has a multifactorial cause, the discordance of blood group antigen expression on the RBCs of donor and patient has been found to be the major cause and limited C-, E-, and K-matched RBC transfusion has been shown to reduce alloimmunization and has been recorded as a consensus in the United States for SCA patients (Chou and Fasano, 2016). Studies have recorded the advantage of matching for E, C, and K to have reduced the rate of alloimmunization from 3% to 0.5% per unit among the chronically transfused SCA cases, which has also been incorporated as a standard practice in Western countries (Vichinsky et al., 2001). Further, extending the prophylactic matching to include RH, KEL, JK, FY, and MNS has also been shown to improve efficacy (Lasalle-Williams et al., 2011). Further, the complexities around RH-compatibility matching among donor and recipient with SCAs can be exaggerated by the presence of mutations and hybrid alleles of *RHD* and *RHCE*, which triggers the formation of RH antigens with missing epitopes which make alloantibodies against the missing epitope on transfusion (Flegel, 2006). The complexity also enhances when the SCA recipient exhibits rare blood group with high incidence antigen wherein they lack the presence of the common antigen expressed in almost all donor RBCs, causing RBC destruction in the recipient upon transfusion (Yazdanbakhsh et al., 2012).

Molecular immunohematology has been reported as a good adjunct to serological testing as molecular analysis among patients alloimmunized to the Rh antigen, even after Rh-matched transfusion revealed presence of altered *RH* alleles. The variability in Rh protein expression levels, alteration in protein folding and conformation on the RBCs, complicates serologic determination of the Rh antigen status (Chou and Westhoff, 2011). Alternatively, the use of automated immunohematology systems which involve use of gel cards are also popularly used for blood typing.

The aim of the present retrospective study includes data analysis of the SCA patients who have undergone antibody screening and have a history of multiple transfusion at different time points from Riyadh, Saudi Arabia, and analyse the facts regarding RBCs transfusion, alloimmunization, effect of Rh-Kell antigen matching units and its impact on SCA patients to decrease the incidence of alloantibody development.

2. Materials & methods

2.1. Study design

The retrospective study includes data from a total of 246 SCA patients inclusive of 141 males and 105 females from the databases from the King Saud Medical City, Regional Laboratory and Central Blood Bank in Riyadh, Saudi Arabia. The samples included for the study involved SCA patients including pregnant women, who have undergone antibody screening test for alloimmunization between year 2010 – 2020, with history of multiple transfusion (>3 times) at different time points. The antibody screening was done by the indirect antiglobulin test (IAT). Ethical approval was obtained from the Institutional Review Board of Central IRB at the Ministry of Health (Central IRB-MoH log No: 2019–0122 M).

2.2. Statistical analysis

The data were analyzed using IBM SPSS (version 25) software. Numerical data were presented as means ± standard deviation and medians, whereas qualitative data were described as frequencies and frequency percentages. Pediatric patients comprised the major cohort and were dichotomized at the respective tradeoff mark by operating characteristic curves. The number of blood transfusions received by the patients varied considerably and was trichotomized with the bearing trend. The chi-square test was used for measuring the association between the development of RBC alloantibodies after the blood transfusion and all the predictors. Backward-step binary logistic regression analysis was applied to ascertain the influence of predictors on the outcome. Both the crude odds ratio and adjusted odds ratio were reported. All the statistical inferences were drawn with 95% confidence intervals, and the ***P values*** were presented up to three decimal places.

3. Results

3.1. Study patient characteristics

Data search for SCA patients who have undergone multiple blood transfusions as well as the indirect antiglobulin test, between the year 2010 – 2020, yielded a total of 246 results inclusive of 141 males and 105 females from the databases of the King Saud Medical City, Regional Laboratory and Central Blood Bank in Riyadh, Saudi Arabia, respectively. The mean age of the study cohort was found to be 11.9 ± 6.7 years, and the median number of transfusion events were found to be six. A total of 52.4% of the study cohort was found to have received blood transfusion matching phenotype; Rh-K. The baseline characteristics have been highlighted in Table 1. The baseline analysis also identified 17.9% of the alloimmunized patients to have received blood transfusion before alloimmunization. In this case, the number of transfusion events cannot be directly linked to alloimmunization events, as the latter is multifactorial and can be impacted upon by dosage, immunogenicity, as well as pre-existing inflammatory disorders.

Further, analysis was also extended to study patient characteristics among those with RBC alloantibodies post blood transfusion. The co-variables considered to identify impacting factors included age, gender, blood group, Rh-D status, number of transfusion events, the Rh-K phenotypes, and phenotype-matching transfusions. This analysis identified, of all transfused, upwards of a month ago, 44.2% to have received their last transfusion and their average lifespan was 8 years at the first transfusion. No association was found between gender and the production of antibodies ($p = 0.73$). A total of 3.72% of the patients were found to be alloimmunized and a significant correlation with advancing age was

Table 1
Baseline characteristics of the study sample (N = 246).

Characteristic	Description	n (%)
Age (yr)	Median (Q1 - Q3)	11 (8–14)
	<14	194 (78.9)
	≥14	52 (21.1)
Gender	Female	105 (42.7)
	Male	141 (57.3)
Blood Group	A -ve	8 (3.3)
	A + ve	50 (20.3)
	AB + ve	5 (2.0)
	B -Ve	5 (2.0)
	B + Ve	25 (10.2)
	O -Ve	12 (4.9)
	O + Ve	141 (57.3)
Number of Transfusions	Median (Q1 - Q3)	6 (3–12)
	<10	170 (69.1)
	10–20	42 (17.1)
	>20	34 (13.8)
Phenotype	Not Recorded	105 (42.7)
	Yes	141 (57.3)
Rh-K Phenotype	ccee K neg	48 (34.0)
	ccEe K neg	14 (9.9)
	ccEE K neg	2 (1.4)
	Ccee K neg	39 (27.7)
	CcEe K neg	19 (13.5)
	CCee K neg	15 (10.6)
	ccee K pos	2 (1.4)
	Ccee K pos	1 (0.7)
	CCee K pos	1 (0.7)
transfusion is matching phenotype (Rh-K)		129 (52.4)
Antibodies		44 (17.9)

found (p = 0.02). Our analysis also identified significant correlation with number of transfusion events (p = 0.003). Total of 12.1% of the patients responded favourably to the Duffy blood group system, 9.9% to ccEe K neg antigens directly, and 1.4% to ccEE K neg antigens. Further, 10.6% patients exhibited favourable response to the Kell blood group mixture of Ccee K neg, CcEe K neg, and CCee K neg antigens. The findings have been summarized in Table 2.

3.2. Alloimmunization correlation

The likelihood analysis for development of alloantibodies among adults (≥14 year), male gender, Rhesus-D (+Ve), blood transfusions events (>7 units, and transfusion matching phenotype (Rh-K) was found to be 2.31, 1.09, 1.67, 3.30, and 0.71 times more than their respective reference groups. Backward step binary logistic regression analysis predicted recipients of blood transfusions of > 7 units to be 3.66 times more exposed to the risk of developing alloantibodies (1.23 – 10.85, 95% CI). On the contrary, transfusion among matching phenotype (Rh-K) were found to be less likely [OR = 0.012 (0.001–0.11, 95% CI)] to develop alloantibodies. The findings have been summarized in Table 3.

The developed alloantibodies among the 17.9% patients, was found to have diminished among 54.5% patients, while among 45.5% it continued to persist. The persistent and unchanged characteristics of the developed alloantibodies was chiefly observed in 'E' phenotype (70%; p = 0.001) and 'small c' phenotype (25%; p = 0.014). Antibody detection analysis found 10.2% of the patients to have responded favourably to the Rhesus blood group scheme, 4.5% to D antigen directly, 40.9% to E antigen, 15.9% to C antigen, and 40.9% to E antigen. Results have been highlighted in Table 4.

4. Discussion

Development of alloantibodies or anti-RBC antibodies is a significant challenge that complicates transfusion therapy among SCA patient's world over, as patients displaying alloantibodies become a part of the high frequency transfusion requirement category, and often end up in immunosuppressive regimen (Spanos et al., 1990; Alkindi et al., 2017). Though RBC-antigen matching has been advocated as a preventive preclude to alloimmunization, there are drawbacks recorded around not all alloantibodies being harmful, the cost of matching, as well as donor feasibility. Alloimmunization reports on SCA, and thalassemia patients have been recorded and one such single centre from Oman, found the rate of alloimmunization to be high at 31.6% among SCA patients, and 20% among thalassemia group. Further, 85% of the patients were immunised with Rh and Kell antigens, and antibodies against E, e, C, c, D, K, S, Fy^a, Kp^a, Jk^a and Cw were observed. This study highlights the need for extended red cell phenotyping among donors and recipients to identify ABO, Rh, and Kell matched transfusion events (Alkindi et al., 2017). The aim of our retrospective study was to evaluate the incidence of alloimmunization among SCA patients following blood transfusions using data from the King Saud Medical City in Riyadh. The focus was to identify factors that increase the risk of formation of anti-RBC antibodies.

In our study, the mean age of patients included was 11.9 ± 6.7 years, and age and number of blood transfusions were found to be significant factors that increased the risk of developing alloantibodies. Further, the average age of the first transfusion was found to be eight years. No association was found between gender of the SCA patients and risk of alloimmunization, but the alloimmunized cohort had higher number of females as compared to males, albeit with no statistical significance. Further, childbirth and pregnancy were also associated with an increased incidence of alloimmunization. Studies have identified RBC alloimmunization to occur in about 30% of SCA patients who receive blood transfusions and about 2–5% of all patients without the disease (Campbell-Lee and Kittles, 2014). Our study found the frequency of alloimmunization to be 3.72% among SCA patients. The risk of alloimmunization among SCA, has been correlated with many individualized susceptible factors including age of the recipient and age at first transfusion, levels of proinflammatory cytokines including IL-1, IL-6, IFN- γ, and white blood cell count (Jison et al., 2004; Belcher et al., 2000). Studies have also found the age of alloimmunized patients to be older compared to the ones negative for the same including one from Brazil, which recorded at recruitment, the median age of alloimmunized to be 24.6 years compared to ones with negative indirect Coomb's test at 11.5 years. This study also found no link between alloimmunization rate and gender (Pinto et al., 2011). Another study among the SCA identified the median age of the alloimmunized group to be 23.3 years, compared to 14.6 years for the non-alloimmunized group (P < 0.0001). Further, number of transfusions (P = 0.00006), older age (P = 0.056) and Hb SC (P = 0.02) were shown to be independently associated with alloimmunization (Murao and Viana, 2005). Our results of this study highlight increasing age to be a significant risk factor for alloimmunization (p = 0.02) among SCA patients; consistent with previous published reports. Our study also identified number of transfusions to be a significant risk factor for alloimmunization wherein recipients with blood transfusions of > 7 units was found to be 3.66 times more exposed to the risk of developing alloantibodies (1.23 – 10.85, 95% CI). A study involving 175 SCA children from French University were studied for RBC alloimmunization, wherein the researchers found the main risk factors to be number of RBC units received (median of 65 vs. 10 units per patient; P = 0.01) and the presence of one or more red cell autoantibodies (46.2% vs. 4.7%; P < 0.0001) (Allali et al., 2017).

Our study finding next also highlights the significance of RBC phenotyping for both donor and the SCA recipient as matching the same Rh-Kell phenotypes before transfusion led to the production of weak significant alloantibodies that diminished and reduced the risk of alloimmunization. Risk of alloimmunization among those where transfusion with matching phenotype (Rh-K)

Table 2
Patient characteristics with respect to development of RBCs alloantibodies post blood transfusion (*N* = 246).

Characteristic	Description	No	Yes	Total	*p* value
Age (yr)	<14	165 (85.1)	29 (14.9)	194 (78.9)	**0.020**
	≥14	37 (71.2)	15 (28.8)	52 (21.1)	
Gender	Female	87 (82.9)	18 (17.1)	105 (42.7)	0.793
	Male	115 (81.6)	26 (18.4)	141 (57.3)	
Blood Group	A -Ve	8 (100.0)	0 (0.0)	8 (3.3)	0.249
	A + Ve	44 (88.0)	6 (12.0)	50 (20.3)	
	AB + Ve	3 (60.0)	2 (40.0)	5 (2.0)	
	B -Ve	5 (100.0)	0 (0.0)	5 (2.0)	
	B + Ve	18 (72.0)	7 (28.0)	25 (10.2)	
	O -Ve	9 (75.0)	3 (25.0)	12 (4.9)	
	O + Ve	115 (81.6)	26 (18.4)	141 (57.3)	
Rhesus-D	-Ve	22 (88.0)	3 (12.0)	25 (10.2)	0.418
	+Ve	180 (81.4)	41 (18.6)	221 (89.8)	
Number of Transfusions	<10	149 (87.6)	21 (12.4)	170 (69.1)	**0.003**
	10–20	29 (69.0)	13 (31.0)	42 (17.1)	
	>20	24 (70.6)	10 (29.4)	34 (13.8)	
ROC-Transfusions	≤ 7	132 (89.2)	16 (10.8)	148 (60.2)	**<0.001**
	>7	70 (71.4)	28 (28.6)	98 (39.8)	
Phenotype	Not done	92 (87.6)	13 (12.4)	105 (42.7)	0.052
	Yes	110 (78.0)	31 (22.0)	141 (57.3)	
Rh-K Phenotype	ccee K neg	35 (72.9)	13 (27.1)	48 (34.0)	0.825
	ccEe K neg	11 (78.6)	3 (21.4)	14 (9.9)	
	ccEE K neg	2 (100.0)	0 (0.0)	2 (1.4)	
	Ccee K neg	32 (82.1)	7 (17.9)	39 (27.7)	
	CcEe K neg	16 (84.2)	3 (15.8)	19 (13.5)	
	CCee K neg	10 (66.7)	5 (33.3)	15 (10.6)	
	ccee K pos	2 (100.0)	0 (0.0)	2 (1.4)	
	Ccee K pos	1 (100.0)	0 (0.0)	1 (0.7)	
	CCee K pos	1 (100.0)	0 (0.0)	1 (0.7)	
Rh-K Phenotype Positivity	No	106 (77.4)	31 (22.6)	137 (97.2)	0.281
	Yes	4 (100.0)	0 (0.0)	4 (2.8)	
Transfusion is matching phenotype (Rh-K)	No	93 (79.5)	24 (20.5)	117 (47.6)	0.306
	Yes	109 (84.5)	20 (15.5)	129 (52.4)	

Table 3
Association between age, gender, Rhesus-D, and blood transfusion.

	Characteristic	Description	Adjusted OR (95% CI)	*p* value	Crude OR (95% CI)	*p* value
Step 1[a]	Age (yr)	<14	–	0.235	–	**0.020**
		≥14	1.99 (0.64–6.23)		2.31 (1.12–4.73)	
	Gender	Female	–	0.920	–	0.793
		Male	1.05 (0.38–2.92)		1.09 (0.56–2.12)	
	Rhesus-D	-Ve	–	0.482	–	0.418
		+Ve	0.59 (0.14–2.54)		1.67 (0.48–5.85)	
	Blood Transfusions	≤ 7	–	**0.049**	–	**<0.001**
		>7	3.09 (1.00–9.54)		3.3 (1.67–6.51)	
	Transfusion is matching phenotype (Rh-K)	No	–	**<0.001**	–	0.520
		Yes	0.013 (0.00–0.11)		0.71 (0.37–1.37)	
Step 5[a]	Blood Transfusions (>7)		3.66 (1.23–10.85)	**0.019**	3.30 (1.67–6.51)	**<0.001**
	Transfusion is matching phenotype (Rh-K)		0.012 (0.001–0.11)	**<0.001**	0.71 (0.37–1.37)	0.520

Table 4
Alloantibodies final status (*N* = 44).

Alloantibodies	Diminished	Persisted	Total	*p* value
	24 (54.5)	20 (45.5)	44 (17.9)	
C	2 (8.3)	5 (25.0)	7 (15.9)	0.217
small c	0 (0.0)	5 (25.0)	5 (11.4)	**0.014**
D	1 (4.2)	1 (5.0)	2 (4.5)	1.000
E	4 (16.7)	14 (70.0)	18 (40.9)	**0.001**
K	9 (37.5)	6 (30.0)	15 (34.1)	0.752
Jk[b]	1 (4.2)	2 (10.0)	3 (6.8)	0.583

was done, were found to be less likely [OR = 0.012 (0.001–0.11, 95% CI)] to develop alloantibodies A recent publication by Campbell-Lee et al., (2018) assessed prevention of alloimmunization in SCA patients post implementation of leukoreduction and prophylactic antigen matching (PAM). This study found alloimmunized patients to have had a higher median number of units transfused compared to non-alloimmunized patients (median of 8 units). Further, combination of leukoreduction and extended antigen matching for alloimmunized patients was found to be significantly associated with reduction in the number of additional RBC alloantibodies detectable after transfusion. Another prospective study involved comparing efficacy of Rh and Kell phenotype matched with conventionally cross-matched blood with incidence of alloimmunization. This study found no alloimmunization in the group

transfused with ABO, Rh and Kell matched phenotypes, while an alloimmunization frequency of 0.4% was noted among the ABO and Rh D matched and transfused group (Makroo et al., 2017).

In Saudi Arabia, the prevalence of hemoglobinopathies have been noted to be high, and alloimmunization has been noted in 13% – 18% of SCA cases, with the most frequently detected antibody classes being Rh, and Kell respectively. Previous studies from Riyadh have addressed to determine the frequencies of Rh and K antigens with their phenotypes among blood donors. One such study identified the D antigen among 86% of the donors, and the most common Rh phenotypes to be R1r at 31%, and R1R1 at 22% respectively, which were found to be different from previous published reports on other population groups including Caucasians, Africans, and Indians, indicating the need for population-specific database. This study also found the e antigen to be the most common Rh antigen at 97.2% (Alalshaikh et al., 2021). Our retrospective study was also focussed at identifying factors, which significantly affect risk of alloimmunization, and our results show older age of>14 years, regular and episodic blood transfusions, and early age of initiation of transfusion to increase the risks of alloimmunization. The risk of alloimmunization, can be reduced through adequate phenotypic antigen matching, especially of the Rh and Kell antigens which can be applied in clinical practices and policies to safeguard SCA patients, improve outcomes, reduce mortality risks, and improve quality of life.

5. Conclusion

The current study showed that advancing age, and multiple transfusion episodes to increase the risk of developing alloantibodies following blood transfusion among SCA patients. The authors recommend the need for future studies involving large patient populations to increase the generalizability and transferability of the results and evaluate the influence of gender, pregnancy, and childbirth on the risk of alloimmunization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are thankful to the Researchers Supporting Project number (RSP-2022R506) King Saud University, Riyadh, Saudi Arabia for funding this work.

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