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Original Article

Transfusion Medicine Section

Antibody Screening, Identification and Red Cell Alloimmunisation Analysis in Multi-Transfused Patients at a Tertiary Care Hospital, Amritsar, India

AKASHDEEP SINGH[1], HARJOT KAUR[2], PARUL GARG[3], SHABAD PREET KAUR[4], BIKRAMJIT SINGH KHURANA[5], JAPNEET KAUR[6], KARAMJIT SINGH GILL[7], DEEPAK VERMA[8]

ABSTRACT

Introduction: Alloimmunisation to red blood cell antigens, resulting from genetic disparities between donors and recipients, is one of the risks associated with blood transfusions. Antibody screening cells are used to detect unexpected antibodies. The risk of alloimmunisation is higher in patients who have undergone multiple blood transfusions.

Aim: To estimate the frequency of various Red Blood Cell (RBC) alloantibodies and to determine the types of antibodies present in repeatedly transfused patients.

Materials and Methods: This cross-sectional study was conducted on 200 patients with a history of multiple blood transfusions from October 1, 2019, to April 30, 2021, at the blood centre of Sri Guru Ram Das Institute of Medical Sciences and Research (SGRDIMSR), Amritsar, Punjab, India. Antibody detection and identification were performed, and the results were recorded. The data was statistically analysed using the Statistical Package for Social Sciences (SPSS) version 26.0 to draw relevant conclusions. The observations were tabulated in the form of numbers and percentages. Categorical data was analysed using the Chi-square test. The level of significance was determined as $p \leq 0.05$.

Results: The study included 200 patients who were on multiple transfusions. The most common blood group among the patients was B positive (39%), followed by O positive (26%). The majority of patients (73.50%) had solid malignancies, followed by 28 (14%) thalassemia patients and 25 (12.50%) patients with chronic kidney disease. Solid malignancies included patients with breast cancer, cervical cancer, ovarian cancer, prostate cancer, and liver cancer. Alloantibodies were found in 15 patients (7.50%), of which 11 had solid malignancies and 4 had thalassemia. The most frequent antibody detected was the anti-K antibody (40%). Alloantibody formation was observed in both males and females. However, no statistical significance was found between gender and alloimmunisation ($p=0.940$).

Conclusion: The effect of alloimmunisation can be avoided by routine RBC antibody screening before blood transfusion, especially in patients with a history of multiple blood transfusions. These measures decrease the incidence of red blood cell alloimmunisation and delayed hemolytic transfusion reactions in multi-transfused patients.

Keywords: Antibody screening cells, Alloimmunization, Genetic

INTRODUCTION

Blood transfusion support is vital for the management of patients with haematologic disorders and malignancies. Many such patients require multiple blood transfusions during their illness or may require them over a lifetime [1]. These patients are prone to haemolytic transfusion reactions resulting from alloimmunisation against red cell antigens. This can be prevented by detecting the exact specificity of the antibody in the patient and providing blood that lacks the corresponding antigen to the patient [2]. Therefore, the detection of alloantibodies present in multi-transfused patients is very important [3].

After the introduction of the Coombs' test, the safety margin for blood transfusion increased considerably [4]. It became possible to rapidly identify the presence of any alloantibody or autoantibody before transfusion. A total of 30 blood group systems have been recognised by the International Society of Blood Transfusion (ISBT). Of these, nine (ABO, Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) blood group systems are considered significant as they are known to cause haemolytic transfusion reactions and haemolytic disease of the fetus and newborn [5,6]. The most pivotal RBC alloantibodies present in daily transfusion practices are directed toward RH (anti-D, -C, -E, -c, and -e), Kell (anti-K), FY (anti-Fya and -Feb), JK (anti-Jka and -Job), and MNS (anti-M, -S, and -s) blood group systems [7]. Of these, the D-antigen is the most immunogenic, resulting in more than 80% of individuals becoming alloimmunised after a transfusion of D-positive packed red blood cells. The antibody screening cells are used to detect unexpected antibodies. These clinically significant antibodies are usually Immunoglobulin G (IgG) antibodies that react at 37°C after incubation or in the AHG phase of the Indirect Antiglobulin Test (IAT).

Pre-transfusion compatibility testing includes checking the ABO group and Rh D type, screening for RBC antibodies, and identifying their specificity. Then, donor RBC units are selected accordingly, which are appropriate for the recipient's ABO and Rh D type for transfusion to the patient. The unexpected antibody status of the donor is also determined before proper cross-matching [8]. Compatibility testing is routinely carried out concerning major blood group antigens, i.e., ABO and Rh antigens [9].

Antibody detection is a key process in pre-transfusion compatibility testing and is one of the principal tools for investigating haemolytic transfusion reactions and immune haemolytic anemias [10]. The antigen-matched transfusion would effectively prevent alloimmunisation. For that, typing of the patient's ABO, Rhesus, Kell, Kidd, and Duffy systems should be done at diagnosis or before transfusion therapy.

Matching should be done at least with ABO, Rhesus, and Kell systems before the transfusion of blood. Furthermore, a

leukocyte filter should be used during the transfusion to prevent alloimmunisation due to white blood cells [11]. Antibody screening at each visit should be done for earlier detection of antibodies. Patients should be encouraged to receive transfusions from only one centre and to use dedicated donors to reduce the alloimmunisation rate [12].

This study was carried out to look at the frequency of alloantibodies in multi-transfused patients who have a higher risk of alloimmunisation.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology and Blood Transfusion, SGRDIMSR, Amritsar, Punjab, India, on patients requiring multiple blood transfusions. The study was done in period from 1st October 2019 to 30th April 2021. A total of 200 cases were included in the study.

Inclusion criteria: Patients with a history of multiple blood transfusions who required transfusion at intervals of 2-4 weeks, those repeatedly transfused patients with difficulty in cross-matching to find matched blood and those patients who had a positive Direct Coombs Test (DCT) and were suspected to have alloantibodies were included in the study.

Exclusion criteria: Patients with known connective tissue disorders and those for whom clinical history and regular follow-up were not possible were excluded.

Procedure

A detailed clinical and transfusion history was obtained using a proforma. The history included the indication for transfusion, the number of blood units transfused, the record of any out-of-group transfusion, and transfusion of any other blood components. The age at splenectomy, if done and drug history were also included. Antibody detection and identification were done.

A working proforma was filled for those who were included in the study. Samples of the patients were collected, and direct Coombs, indirect Coombs, antibody screen, and antibody identification were done by the column agglutination method. Three cell antibody screening was performed using anti-human globulin gel cards (ID-Card LISS/Coombs) and a three-cell panel (ID-Dia Cell I, II, III). Those with positive antibody screening were further analysed for the antibody identification test using an eleven-cell panel (Set ID-Dia Panel).

STATISTICAL ANALYSIS

Data from the present study were statistically analysed using SPSS version 26.0 to draw relevant conclusions. The observations were tabulated in the form of numbers and percentages. Categorical data were analysed using the Chi-square test. The level of significance was determined as p≤0.05, considered significant, and p≤0.001, considered highly significant.

RESULTS

Out of 200 cases, 145 (72%) were females and 55 (28%) were males [Table/Fig-1]. The most common age group was between 41-60 years, with 104 patients (52%), and the least common age group was 0-20 years (28 cases) [Table/Fig-1]. The most common blood group among the patients was B Positive (39%), followed by O Positive (26%), and the least common was B negative (1%) [Table/Fig-2]. The maximum number of patients (147, 73.5%) had solid malignancies, followed by thalassemia patients (28 cases, 14%), and the least had chronic kidney disease (25 cases, 12.5%). Among solid malignancies, 39.50% were of Carcinoma (Ca) Breast, 14% of Ca Cervix, 11% of Ca Ovary, 6% of Ca Prostate, and 3% of Ca Liver [Table/Fig-3]. In the present study, alloantibodies were found in 15 patients, accounting for

Age (years)	Males 56 (28%)	Females 144 (72%)	Total	Percentage
0-20 years	8	20	28	14
21-40 years	8	21	29	14.5
41-60 years	29	75	104	52
≥61 years	11	28	39	19.5
Total	56	144	200	100

[Table/Fig-1]: Distribution of patients according to age group and sex (N=200).

Blood group	No. of cases	Percentage
A+ve	36	18%
A-ve	6	3%
B+ve	78	39%
B-ve	2	1%
O+ve	52	26%
O-ve	4	2%
AB+ve	3	2%
AB-ve	19	10%
Total	200	100%

[Table/Fig-2]: Distribution of patients according to blood group (N=200).

7.50% of patients [Table/Fig-4]. Alloantibody formation was observed in both males and females. However, no statistical significance was found between gender and alloimmunisation. The p-value between gender and alloimmunisation was 0.940 [Table/Fig-5]. Out of the 15 patients, the maximum (10 patients) were from the age group of 41-60 years, and the least (01 in number) were in the age group of 21-40 and ≥61 years of age [Table/Fig-5].

Diagnosis	N=200		Percentage
Thalassaemia		28	14%
Solid maligncies	Ca cervix	28	14%
	Ca breast	79	39.50%
	Ca prostate	12	6%
	Ca liver	6	3%
	Ca ovary	22	11%
Chronic kidney disease		25	12.50%
Total		200	100%

[Table/Fig-3]: Distribution of patients according to their diagnosis (N=200).

Parameter	Males	Females	Total	Percentage	Chi-square	p-value
Non-alloimmunised	51	134	185	92.50%	0.005	0.940
Allo-immunised	4	11	15	7.50%		
Total	55	145	200	100%		

[Table/Fig-4]: Gender distribution in alloimmunisation (N=200).

The most frequent antibody detected was anti-K antibody, found in six out of 15 patients (40%), followed by anti-C, seen in 4 (26.67%). anti-E was the least common antibody detected [Table/Fig-6]. In thalassemia patients, anti-K antibody was found in three out of four allo-immunised patients, and anti-E antibody was found in one out of four allo-immunised patients [Table/Fig-6]. In patients with solid malignancies, anti-C antibody was found in 04 patients (26.67%), anti-K and anti-M antibody were found in 03 patients (20%) each, and Anti-E antibody was found in 02 patients (13.33%) [Table/Fig-6]. There was no statistically significant association between the number of packed RBC units transfused and alloimmunisation (p=0.528). Therefore, in this study, the association of the number of packed RBC units transfused as a risk factor for alloimmunisation was not established [Table/Fig-7].

Age (years)	Total no of cases	Non-alloimmunised	Percentage	Alloimmunised	Percentage	Chi-square	p-value
0-20	28	25	12.5	3	1.5	3.143	0.370
21-40	29	28	14	1	0.5		
41-60	104	94	47	10	5		
≥61	39	38	19	1	0.5		
Total	200	185	92.5%	15	7.5%		

[Table/Fig-5]: Age distribution in alloimmunisation (N=200).

Serum antibody detection		No of cases				
		Anti-K	Anti-C	Anti-M	Anti-E	Total
Thalassaemia		3	0	0	1	4
Solid malignancies	Ca cervix	1	0	0	0	1
	Ca breast	0	2	1	1	4
	Ca ovary	1	0	1	0	2
	CML	0	1	1	0	2
	Ca liver	0	1	0	0	1
	Multiple myeloma	1	0	0	0	1
Total		6	4	3	2	15
Percentage %		40	26.67	20	13.33	100

[Table/Fig-6]: Type of antibody detected in patients.

No of transfusions	No. of cases	Non-Alloimmunised	Alloimmunised
<5	111	104	7
5-10	62	57	5
11-15	4	4	0
16-20	0	0	0
≥21	23	20	3
Total	200	185	15

[Table/Fig-7]: Number of PRBCs transfusions and alloimmunisation.

DISCUSSION

Blood transfusions, although often a beneficial and potentially life-saving treatment for those with severe anaemia, are not without risks. Historically, infections transmitted through transfused blood have been a major concern. However, with the implementation of much more sophisticated and stringent screening techniques, the risks of infection have drastically decreased. Emerging evidence has indicated, however, that non-infectious serious hazards of transfusion may be associated with a significantly higher rate of adverse events linked to transfusion than previously appreciated [13,14].

On a daily basis, pre-transfusion testing is done to prevent immune-mediated hemolytic transfusion reactions. The steps of pre-transfusion testing involve reviewing the acceptability of blood samples, checking the ABO group and Rh D type, choosing suitable donor RBC units for recipients, and carrying out a cross-match. As blood is routinely matched in relation to major blood group antigens, i.e., ABO and Rh D antigen, the probability that the donor will have minor blood group antigens not present in the recipients, which will result in alloimmunisation, is high [15].

Out of 200, 15 were alloimmunised, of which 11 were females and four were males. The p-value between gender and alloimmunisation was not statistically significant (p=0.940). Although female gender has been identified as a risk factor for alloimmunisation in the general population [16,17], the predominance of female subjects in alloimmunised individuals can be explained by the antigenic stimulation due to foetal red cells during pregnancy, but perhaps also to other unknown factors. However, the studies done by Thompson AA et al., (p=0.562), Bhuva DK and Vachhani JH (p=0.912), Sood R et al., (p=0.557), and El Danasoury AS et al., (p=0.007) showed no statistically significant association between gender and alloimmunisation [18-21].

In the present study, three alloimmunised patients were less than 20 years of age, 1 alloimmunised patient was from the 21-40 years age group, 10 alloimmunised patients were from 41-60 years of age group, and 1 alloimmunised patient was in the above 61 years category. The p-value between age and alloimmunisation was not statistically significant (p=0.370). This non-significance may be the result of the small number of alloimmunised patients.

Although younger age at the time of initial RBC exposure correlates in some studies with a lower likelihood of alloimmunisation [22,23], the immunologic mechanisms behind these observations are not fully understood. Several hypotheses have been evoked to explain these findings:

- A lower ability to produce antibodies in young children due to immunological immaturity.
- Induction of tolerance to erythrocyte antigens by repeated early transfusions [24].

These findings are not unique to patients with thalassaemia, with similar trends reported in patients with Sickle Cell Disease (SCD). However, a study conducted by Ben Amor I et al., about thalassaemia and sickle cell patients supports these findings [25]. They reported a lower rate of alloimmunisation in thalassaemia patients whose mean age is lower than that of SCD patients despite a higher consumption of red blood cell concentrates.

There was no significant relation between sex and the risk of immunisation, nor between the number of red cell units transfused and alloimmunisation. On the other hand, there was a significant relation between autoimmunisation and the number of red cell units transfused in thalassaemia (p<0.001) [25]. In another study in the United States, 29% of transfused SCD children developed alloantibodies versus 47% of SCD adults [26]. However, some studies have not found a significant difference between age at transfusion initiation and alloimmunisation. The median age at first transfusion was the same for alloimmunised and non-alloimmunised patients (3.0 years; p-value=0.91) [27].

In the present study, the number of packed cell units transfused ranged from 2 to 360 units with a mean number of 16.2 units. In thalassaemia patients, the mean number of transfusions was 92.2. However, in solid malignancies, the mean number of units transfused was 4.38.

In this study, the mean number of units transfused was 20.73 units, ranging from 3 to 150 units. The p-value calculated was 0.528. Hence, no statistically significant association was seen between the number of packed cell units transfused and the risk of alloimmunisation.

Similar results were seen in the study done by Sood R et al., (0.30%) and Bhatti FA et al., [20,28]. Alloimmunisation in patients having multiple transfusions was seen in 7.5% of cases. This was in concordance with the study done by Pimpaldara RP et al., in which the rate of alloimmunisation was 7% [15]. However, studies by Shastry S et al., reported a low rate of alloimmunisation, which was 4.8% [29]. This low rate of alloimmunisation was explained by homogeneity between the donor and the recipient population [30].

The rate of alloimmunisation in thalassaemia was 10.71% i.e., (3 out of 28 patients). Similar results were observed by Gupta R et al., and Pradhan V et al., [31,32]. In the present study, the rate of alloimmunisation in solid malignancies was 8.16% (12 out of 147 patients). This is in concordance with the study done by Mohsin S et al., who found 6% alloimmunisation in non-haematological malignancies [33]. No alloantibody was found in CKD patients. This may be due to a very small study population.

The specificity of most alloantibodies detected in the present study was against Rh and Kell antigen systems. This may be due to their high immunogenicity, which is in concordance with previous studies [34,35]. In the present study, Rhesus and Kell blood groups comprised the most alloantibodies with 40% each, followed by anti-M with 20%. Among the Rh blood group, anti-C was 26.67%, the most common, followed by anti-E with 13.33%.

However, in thalassaemia, anti-K comprised the major antibodies, three out of four i.e., 75%, followed by anti-E, which showed similar results with Roopam J et al., with an 80% incidence of anti-K. A higher rate of anti-K alloantibodies could be explained based on the higher number of Kell positive antigens in the donor population of our region [35]. A study carried out by Beshlawy EA et al., in Egypt concluded that in thalassaemia patients, due to multiple transfusions, alloimmunisation and autoimmunisation are commonly seen. The most frequent alloantibodies detected were against Kell, Rh, Lutheran, and Lewis systems [36].

In patients with solid malignancies, antibodies against the Rh blood group were in five out of 11 i.e., 45.45%, followed by anti-M and anti-K in three out of 11 i.e., 27.27%, which was comparable with Mohsin S et al., who found 55.55% of patients with non-haematological malignancies developed alloantibodies against the Rh system. Genetics and race seem to influence the development of alloantibodies, which might have affected the higher occurrence of developing anti-Rh antibodies observed in the present study [37]. Hence, transfusion of blood matched for Rh and K antigens will prevent alloimmunisation.

Limitation(s)

Haemoglobinopathies, except for thalassaemia, could not be included in the study. Many diseases like chronic anaemias, chronic liver diseases, and haematological malignancies were not included as the authors didn't get any cases in the present study period.

CONCLUSION(S)

Red cell alloimmunisation should not be ignored in multi-transfused patients. Routine RBC antibody screening should be done before blood transfusion, especially in patients with a history of multiple blood transfusions, to prevent the effect of alloimmunisation. Regular screening of red blood cell alloantibodies in multi-transfused patients would facilitate superior management of the patients. The patient should be given blood after the screening of the antibody. If the antibody is present, then blood should be given accordingly. These measures can facilitate decreasing the incidence of red blood cell alloimmunisation and delayed haemolytic transfusion reactions in these multi-transfused patients.

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PARTICULARS OF CONTRIBUTORS:
1. Consultant Pathologist, Department of Pathology, Krsnaa Diagnostics Pvt Ltd Ludhiana, Punjab, India.
2. Professor, Department of Pathology, SGRDIMSR, Amritsar, Punjab, India.
3. Associate Professor, Department of Immunohematology and Blood Transfusion, GGS Medical College, Faridkot, Punjab, India.
4. House Surgeon, Department of Pathology, Lord Mahavir Civil Hospital, Ludhiana, Punjab, India.
5. Junior Resident, Department of Pathology, Mgm Medical College And Lsk Hospital, Kishan Ganj, Bihar, India.
6. Junior Resident, Department of Pathology, SGRDIMSR, Amritsar, Punjab, India.
7. Professor and Head, Department of Pathology, SGRDIMSR, Amritsar, Punjab, India.
8. Intern, Department of Pathology, SGRDIMSR, Amritsar, Punjab, India

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Parul Garg,
H. No. 34, GGS Medical College Campus, Sadiq Road, Faridkot-151203,
Punjab, India.
E-mail: parultayal45@gmail.com

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