Red cell alloimmunization in multitransfused patients and multiparous women

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Abstract

Purpose To determine the incidence of alloimmunization against red cell antigens and the thermal amplitude and specificity of antibodies in multitransfused patients and multiparous women.

Methods Antibody screening was performed in 30 non-transfused orthopedic surgery cases (control), 504 multitransfused patients and 325 multiparous women. Antibody screening at 4°C, 22°C and 37°C was carried out in a saline phase, by indirect antiglobulin technique (IAT), using papain cystein, low ionic strength solution (LISS) and polyethylene glycol (PEG).

Results In multitransfused patients IgM antibodies were more frequently detected at 4°C and the IgG antibody incidence was 7.1% by enzyme method, 7.7% IAT, 9.4% LISS, 10.2% using PEG & 10.2% multiparous women. Bad obstetric history cases had significantly higher incidence of alloimmunization. The antibody specificity of antibodies was mainly in Lewis, Rh, Kidd and MN systems.

Conclusion Antibody screening before transfusion, at set time intervals after transfusion and during antenatal period is recommended.

Keywords Red cell alloimmunization · Multiparous women · Multiple transfusions · Antibody specificity · Thermal amplitude · Polyethylene glycol (PEG) · Low ionic strength solution (LISS)

Introduction

Multitransfused patients suffering from β -thalassemia, sickle cell disease (SCD), chronic renal failure (CRF), etc. need frequent blood transfusions thus facing a constant risk of alloimmunization [1, 2]. Similarly multiparous women and those having bad obstetric history (BOH) also have greater chances of alloimmunization to red cell antigens [3, 4]. Present study was undertaken in selected groups of multitransfused patients to evaluate their ability to generate blood group antibodies. Multiparous women were also investigated to find out the incidence of red cell alloantibodies. In this study, thermal amplitude and specificity of antibodies was also determined.

Materials and methods

Before starting the project it was discussed in the Institutional Ethics Committee and their approval was obtained. In the present study 504 patients receiving multiple transfusions for severe anemia or blood loss were selected. All patients had received more than six units of blood. Healthy patients who were scheduled for an orthopedic surgery and never received blood transfusion before were selected as the control group. All multitransfused patients' blood was collected prior to transfusion of whole blood or packed red blood cells. Those who received more than six transfusions at a time for the first time in life were called for antibody detection three to six months after the transfusions. Study

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also included 350 women having more than one pregnancy but never received any transfusion.

Data on age, sex, transfusion or obstetric history, etc. were collected in a proforma. Five ml of blood was collected in EDTA and plain vials. Investigations such as ABO and Rh (D) grouping, antibody screening with pooled O cells in saline phase at 4°C, 22°C and 37°C, enzyme (1% papain cystein) and indirect antiglobulin test (IAT) were carried out [5–7]. Low ionic strength solution (LISS) [6] and 20% solution of polyethylene glycol 6000 (PEG) [8] were used to enhance the antigen antibody reaction. Auto control was kept for every serum sample. Samples found reactive in the above antibody screen were tested for identification of antibody specificity using DiaMED-ID panel of 11 red cells and saline and IAT techniques.

Results

The study includes 504 multitransfused patients among which 296 were males and 208 females. Age of the patients varied from newborn to >60 years. The mean age of patients was 31.5 ± 12.2 years. Thalassemia and SCD patients were of lower age group as they start with transfusion early in their life. In multiparous women the parity ranged from 2 to 7 and only one woman was investigated during seventh pregnancy.

Out of the 504 patients, 72 (14.3%) showed the presence of saline antibody. Some antibodies were biphasic reacting at two different temperatures. The frequency of clinically non-significant cold antibodies was higher in multitransfused compared to multiparous women. Seven cold antibodies were auto antibodies. Table 1 shows the antibodies detected at different temperatures. Enzyme technique had overall incidence of 19.6% in multitransfused and 15.5% in multiparous women. Statistical evaluation between multitransfused and multiparous women by χ^2 test did not reveal any significant difference in antibody formation incidence ($p > 0.1$).

Table 2 illustrates antibody detection in patients with respect to diagnosis. In the control group there was no antibody formation. Some atypical antibodies which did not react in AHG phase were detected using LISS and PEG. PEG

was the most sensitive technique. But the χ^2 test showed no association between antibody detection in patients of different groups using different media or antibody detection by different techniques ($\chi^2 = 7.76$ for 16 degrees of freedom, $p > 0.1$). Statistical comparison of immuno-compromised patients receiving multiple transfusions over a long period and subjects receiving multiple transfusions at a time did not show significant difference ($p > 0.1$).

Table 3 presents the incidence of antibody formation with respect to number of pregnancies. It shows that as the number of pregnancies increased the incidence of antibody formation increased. But the χ^2 test did not show significance among different groups ($p > 0.1$) probably because of the small numbers.

Table 4 presents the antibody formation data of 325 multiparous women having normal pregnancies and those who had had obstetric history (BOH). Statistical evaluation showed significantly higher incidence of antibody production in BOH compared to normal pregnancies. ($\chi^2 = 42.1$ for 3 degrees of freedom, $p < 0.001$).

Total 46 serum samples giving strong reaction in thermal amplitude test or AHG phase were further tested for specificity using commercially available DiaMed cell panel. The antibody specificity of cold antibodies was anti-I, -Le^a, Le^b and anti-N and warm antibodies were -C, -e, -c, -Jk^a, Lu^b, -S, -s, -M, -Xg^a. Some antibodies such as -I, -Jk^a, -M, -c, -S, Le^b were biphasic in nature.

Discussion

Blood banks provide ABO and Rh (D) antigens matched blood. RBC alloimmunization results from disparity of antigens between donor and recipient. Recipient's immune status, immunogenicity of the antigen and dose of the antigen are the factors which play a significant role in alloimmunization [9]. If the recipient is immuno-compromised at the time of first transfusion then the chances of alloimmunization are less. Spanos et al. [10] observed lower incidence of alloimmunization when thalassemic children started transfusion before three years of age. Higher rate of antibody formation was reported by Stiegler et al. [11] in myelodysplastic syndrome patients having hypersensitive

Table 1 Detection of antibodies according to their thermal amplitude

Series	No. tested	Saline			Enzyme		
		4°C	22°C	37°C	4°C	22°C	37°C
Multitransfused patients	504	41	15	30	41	22	36
%		8.1	3.0	6.0	8.1	4.4	7.1
Multiparous women	325	14	8	14	19	11	21
%		4.3	3.2	4.3	5.8	3.3	6.4
Control	30	0	0	0	0	0	0
		0	0	0	0	0	0

Table 2 Red cell antibody detection by IAT wrt diagnosis

Diagnosis	IAT	IAT LISS	IAT PEG
Thalassemia (125)	11 [8.8]	8* [9.4]	9*[10.6]
Sickle cell disease (12)	1[8.3]	1[8.3]	1[8.3]
Renal disorder (73)	6[8.2]	5**[14.3]	5**[14.3]
Liver disorder (40)	3[7.5]	3[7.5]	3[7.5]
Surgery (84)	6[7.1]	7[8.3]	8[9.4]
Bleeding disorders (51)	3[5.9]	6[11.8]	7[13.7]
Burns (18)	1[5.6]	1[5.6]	1[5.6]
Cancer (10)	1[10]	1[10]	1[10]
Miscellaneous (91)	7 [8.8]	8 [9.9]	10 [11.0]
Total	39/504	40/426	45/426
%	7.7	9.4	10.2
Control orthopedic surgery (30)	0	0	0

Figures within () indicate number of cases; wrt: With respect to;
 Figures within [] indicate % of antibody positive cases

*Only 85 thalassemics tested with LISS and PEG

**Only 35 renal disorder cases tested with LISS and PEG

Table 3 Parity-wise incidence of antibody formation

Multiparous women	Para			
	≤ II	III	IV	Total
Total cases	293	21	11	325
IgG antibody positive	28	3	2	33
%	9.6	14.3	18.2	10.2

Table 4 Incidence of antibody formation in normal and bad obstetric history (BOH) cases

	Normal	H/O Abortion	H/O Stillbirth	H/O Neonatal death
Total cases	263	45	9	8
IgG antibody positive	10	17*	3*	3*
%	3.6	37.7	33.3	37.5

*Statistically significant increase in incidence of antibody formation in BOH cases compared to normal pregnancies

immune status. In our study different categories of patients had statically comparable rate of alloimmunization.

Patients suffering from genetic disorders such as β -thalassemia, SCD or patients having chronic anemia due to kidney failure, need regular transfusions. While in surgery cases or in cases of burns, it may be the first transfusion episode. Though our study showed slightly lower frequency of antibody formation in surgery and burns cases compared to repeat transfusion cases there was no statistically significant difference in these two categories.

In our study 8.8% of β -thalassemia major patients who received saline-washed red cells produced IgG antibodies. The literature shows 5.3% to 8.6% incidence of alloantibody formation in β -thalassemia patients from Asian countries [12–14]. Transfusion of leukoreduced red cells can decrease the RBC alloimmunization [15] and as major-

ity of thalassemia patients receive leukoreduced products the antibody formation rate is comparatively low. SCD patients require blood when there is a sickle cell crisis. Murao and Viana [16] observed 9.9% alloimmunization in SCD. Domen and Ramirez [17] have reported low rate (6.1%) of antibody formation in CRF patients undergoing dialysis while a north Indian study [1] reports 9.9% rate of antibody formation in the same category of patients. Our study detected antibodies in 6 out of 73 (8.2%) CRF patients.

In multiparous women, the rate of antibody production was directly proportional to the increase in parity which may be due to the increased risk of fetomaternal leak with the increase in number of pregnancies. But the χ^2 test did not show significance among different groups ($p > 0.1$) probably because of the small numbers. In normal pregnancy

there were less chances of antibody production (3%) than in BOH cases which is an expected observation [5].

Sensitivity of antibody detection is dependent on the technique employed. LISS and PEG are known to enhance antigen antibody reactions. However there is a risk of getting false positive results because of which blood bank may unnecessarily deny blood to critically ill patients. The specificity of cold antibodies detected in this study was within I, Lewis and MN blood group systems. Among clinically significant antibodies there were Rh specific (anti-C, anti-c and anti-e) and JK^a antibodies. In western countries besides the Rh specific antibodies, Kell antibodies are most frequently detected because 10% of Caucasian population has K antigen. A multivariate analysis of 1710 immunized patients, reported 541 cases (24.9%) having anti-Kell antibody [9]. In India only 0.3% to 0.7% population has K antigen [6], hence chances of detecting this antibody are remote. Our study as well as other study from north India [18] did not have any cases of Kell antibody. But Shukla and Chaudhary [1] have reported Kell antibody in a CRF patient who also had anti-E. In our opinion antigen match cross-match for Kell antigen is not necessary in India. Matching for antigens other than ABO and Rh groups will increase the time necessary for grouping/X matching, as in India there is no RBC antigen database available for the population. As morbidity and mortality due to these antibodies is rare antigen matching for other than ABO and Rh group is not recommended, which is also not cost effective.

Conclusions

Transfusion should be avoided so that the patient is not exposed to foreign red cell antigens thus preventing the risk of alloimmunization. Antibody screening should be carried out at time intervals in patients receiving regular blood transfusions and antenatal women so that the patients having RBC antibodies are managed properly.

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