Rh and Kell Phenotype Matched Blood Versus Randomly Selected and Conventionally Cross Matched Blood on Incidence of Alloimmunization

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Abstract There is paucity of literature regarding efficacy of transfusion of Rh and Kell matched blood in reducing alloimmunization risk among non-chronically transfused patients. A prospective study to compare efficacy of Rh and Kell phenotype matched blood over randomly selected and conventionally cross-matched blood on the incidence of alloimmunization in patients undergoing cardiac surgery was carried out in the Department of Transfusion Medicine at Indraprastha Apollo Hospitals, New Delhi, from 1st September, 2013 to 31st December, 2014. Two groups, A and B of 250 each were studied. Group A received ABO, Rh and Kell phenotype matched units. Group B received units matched only for ABO and Rh D. Retrospective analysis for antigenic exposures was done. Alloimmunization rate was evaluated for both groups after 72 h and 4 weeks and compared. A p value ≤ 0.05 was considered statistically significant. None of the patients in Group A were alloimmunized. In Group B, 119 patients received antigenic stimulus (single antigen stimuli- 93; multiple- 26). The probability of a patient being exposed was 52.4 %. At 6 weeks post transfusion, one patient developed ‘Anti-E’ and had received ‘E’ stimulus once. The rate of alloimmunization was 0.4 % in group B, 0.8 % overall and the risk of alloimmunization per unit transfused was 0.17 %. Non responders were 99.16 %. The study did not reach statistical significance ($p = 0.238$). Majority of our population are non-responders therefore, the resources and time can be reserved for providing Rh and Kell matched units for multiply transfused patients.

Keywords Alloimmunization · Cardiac surgery · Phenotype matching

Introduction

Provision of safe blood is the prime responsibility of every transfusion facility. Testing it for infectious pathogens along with prevention of haemolytic transfusion reactions due to alloimmunization is of prime importance [1]. Efforts at improving the blood safety led to incorporation of antibody screening and identification protocols to identify the exact specificity of the antibody (if any) and provide the corresponding antigen negative blood [1].

With longer life expectancy, the number of chronic degenerative diseases and complex surgical procedures increase, leading to increased demand for blood transfusions. Thereby, increasing the probability of developing RBC alloantibodies [2].

As a result, pre-transfusion antibody screening on patients’ samples has become an essential component of compatibility testing in many countries. However, it still needs to be initiated in many centers in India. A policy of transfusing Rh and Kell matched blood to prevent the development of alloantibodies has been repeatedly proposed and limited to patients with chronic diseases or those who require multiple transfusions. [3] There is paucity of literature with regards to efficacy of this policy in reducing alloimmunization risk among non-chronically transfused patients.

We identified patients undergoing cardiac surgeries at our center as one of the largest and most accessible non-chronically transfused group, as well as a procedure that is associated with one of the highest rates of transfusion ranging from 11 to 97 % [4].

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Thus, we designed the present study to compare the efficacy of providing Rh and Kell matched blood instead of conventionally cross matched blood (i.e. ABO and Rh D specific, cross-matched in AHG phase) on the incidence of alloimmunization in patients undergoing cardiac surgery at our center.

Materials and Methods

A prospective study to compare the efficacy of Rh and Kell phenotype matched blood over randomly selected and conventionally cross-matched blood on the incidence of alloimmunization in patients undergoing cardiac surgery was carried out in the Department of Transfusion Medicine at the Indraprastha Apollo Hospitals, New Delhi, from 1st September, 2013 to 31st December, 2014. Consent for transfusion and enrollment into the study was obtained from the patients in the pre-operative period. Study was approved by the ethical committee.

Inclusion Criteria

Patients admitted in the hospital for undergoing cardiac surgery including but not limited to coronary artery bypass grafts (CABG), valve and aneurysmal repairs.
Pre-operative antibody screening negative.
Received at least one transfusion in peri-operative period.

Exclusion Criteria

Pre-operative antibody screen positive.
History of blood transfusions.
No transfusion during peri-operative period
Intra operative deaths.
Redo and complex cardiac surgeries.
Immunosuppressed patients.
Patients with other chronic diseases or hemoglobinopathies
Immediate post-operative deaths.
Loss to follow up.
Received group nonspecific blood.
Paediatric patients

All patients satisfying the inclusion and exclusion criteria were divided into two groups, A and B each having 250 consecutive patients.

Donor Apart from all routine investigation on donor units, the donor units are typed for ABO, Rh (D, C, c, E, e) and Kell (K) antigens and the results are compiled and maintained.

The ABO and Rh D typing; Rh and Kell phenotyping; Crossmatch, antibody screening and identification is

performed on NEO (Immucor, Norcross GA), fully automated immunohematology analyzer which uses Solid Phase Red Cell Adherence (SPRCA) assay.

Group A

Patients in Group A were typed for ABO and Rh D. Antibody Screening was then performed followed by Rh (C, c, E, e) and Kell (K) phenotyping. After having the phenotyping results, our donor typing records were searched for compatible red cell unit as the patients with respect to ABO, Rh (D, C, c, E, e) and Kell (K) antigens and cross matching was performed in Anti Human Globulin (AHG) phase and kept ready for issue.

Group B

Patients in Group B were also typed for ABO and Rh D. Antibody Screening was then performed followed by Rh (C, c, E, e) and Kell (K) phenotyping. Random ABO and Rh D matched units were selected from inventory and crossmatched in AHG phase and kept ready for issue.

Subsequently, the donor phenotyping records were sought and a retrospective analysis of the transfusion records of the patients was done to note the number of ABO and Rh D matched but Rh (C, c, E, e) and Kell mismatched transfusions. Among the mismatched transfusions, the number of patients receiving stimulus for single antigen and the number of patients receiving stimulus for multiple antigens of Rh and Kell system were noted.

Post Transfusion Investigations

After 72 h [5] and 4 weeks of the last transfusion, the antibody screen was performed for clinically significant antibodies. If the antibody screening result was positive, antibody identification was done to determine the specificity.

Blood Requirement

The number of packed red cells transfused for all the study patients were noted and Crossmatch:Transfusion (C:T) ratio calculated.

Statistical Analysis

For the purpose of statistical analysis, data was collected and entered in the Microsoft Excel (Redmond, WA) and SPSS software (SPSS inc., Chicago, IL) was used for analysis. Descriptive variables were analysed using mean, median, range. Chi square test/Fisher exact test was used wherever required. Wilcoxon rank sum test with continuity

correction was done to show the statistical comparison of both group A and B in terms of patient demographics. A p value ≤ 0.05 was considered statistically significant.

Results

Total number of patients screened for presence of antibodies was 783, of which 283 were excluded from the study. Of the remaining 500, first consecutive 250 patients were assigned group A and the next 250 in group B (Fig. 1).

Patient Demographics

The study patients comprised of 365 males (73 %) and 135 females (27 %). Significant obstetric history was present in 73 and 52 of the females in Group A and B respectively. Overall, the mean age of the study patients was 57.2 ± 12.3 years. Mean age in Group A was 57.1 ± 12.3 years while in group B was 57.3 ± 12.3 years. Both the groups were comparable in terms of demographic data ($p = 0.695$).

Distribution of Rh (C, c, E, e) and Kell (K) Phenotypes

The distribution is shown in Tables 1 and 2. The most prevalent Rh phenotype was DCCee ($n = 230$, 46 %) followed by DCcee ($n = 164$, 32.8 %). Kell negative phenotype was seen in 98.8 % of the patients.

Table 1 Group wise Rh phenotype distribution

Rh phenotype	Group A	Percent (%)	Group B	Percent (%)
DCCEe	1	0.4	0	0
DCcEe	23	9.2	33	13.2
DccEe	12	4.8	6	2.4
DCCee	129	51.6	101	40.4
DCcee	72	28.8	89	35.6
Dccee	1	0.4	7	2.8
dccee	11	4.4	12	4.8
dCcee	1	0.4	2	0.8

Table 2 Group wise Kell phenotype distribution

Kell (K)	Group A	Group B	Percent (%)
Positive	2	4	1.2
Negative	248	246	98.8

Packed Red Cell (PRC) Utilisation in Cardiac Surgery

The number of PRC units transfused, ranged from 1 to 9 units with a mean of 2.3 ± 1.3 and median of 2 units. As per the Maximum Surgical Blood Ordering Schedule (MSBOS) of our hospital, every patient undergoing cardiac surgery is cross-matched for 4 PRC units. Additional PRC units were arranged subsequently, if required. In all, 2063 units were crossmatched and 1155 units were transfused in

Fig. 1 Flow chart showing the summary of the work done

the study patients. This yielded a crossmatch to transfusion ratio (C:T) of 1.8.

Frequency of Rh (C, c, E, e) and Kell Antigens

The most frequent antigen in the study patients was ‘e’ (100 %) followed by ‘C’ (90.2 %). The least frequent antigen was ‘K’ (1.2 %) (Table 3).

Exposure to Antigen Stimulus

In group A, all the patients received Rh and Kell matched units for transfusion hence none of the patients were exposed to these antigens.

In group B, since all the patients were given units matched only for ABO and Rh (D) antigens, the frequency of Rh (C, c, E, e) and Kell antigenic exposure in patients lacking the corresponding antigens were observed (Table 4).

The maximum antigenic exposure was seen with ‘C’ antigen (52 %) followed by antigen ‘E’ (39.3 %) while minimum exposure was noted for ‘K’ antigen (5.3 %). None of the patients received any antigenic stimuli for ‘e’ antigen.

Alloimmunization

Group A

On follow up, no case of alloimmunization was noted after 72 h and 4–6 weeks of last transfusion event.

Table 3 Antigen frequency distribution

Antigen	Patients positive for the antigen (N)	Frequency(%)
C	451	90.2
c	269	53.8
E	75	15
e	500	100
K	6	1.2

Table 4 Antigenic exposures in Group B

Antigen	Antigen positive (N, %)	Antigen negative (N, %)	Exposure in antigen negative	Single exposure	Multiple exposures	Alloimmunized patients	Non responders (%)
C	225 (90 %)	25 (10 %)	13 (52 %)	4	9	0	100
c	149 (59.6 %)	101 (40.4 %)	23 (22.8 %)	10	13	0	100
E	39 (15.6 %)	211 (84.4 %)	83 (39.3 %)	66	17	1	98.8
e	250	0	—	—	—	—	—
K	4 (1.6 %)	246 (98.4 %)	13 (5.3 %)	13	0	0	100

Group B

Total numbers of units transfused were 591 among which 413 were Rh and Kell compatible units and 178 (30.1 %) were unmatched units.

Incidentally, 131 patients out of 250 received Rh and Kell phenotypes that were either exactly matched with the patients’ phenotype or could be considered compatible since they did not expose the patient to any alloantigens. In other words, 119 patients received one or more antigenic stimulus. Of these, 93 were exposed to a single alloantigen while 26 received stimuli from multiple antigens of Rh and Kell system. The probability that a patient was exposed to antigenic stimuli was 52.4 %.

No alloimmunization detected at 72 h. However, at 4–6 weeks post transfusion, one patient developed ‘Anti-E’. This particular patient had received ‘E’ antigen stimulus only once in a single transfusion event.

Overall, the rate of alloimmunization was 0.4 % in group B and the risk of alloimmunization per unit transfused was 0.17 %.

Comparison of Alloimmunization Rates Among the Groups

Alloimmunization was observed in a single patient in Group B, while none developed in Group A. The efficacy of providing Rh and Kell phenotype matched blood units over randomly matched red cell transfusions in preventing alloimmunization in patients undergoing cardiac surgeries was not statistically significant ($p = 0.238$).

Discussion

Donor typing for Rh and Kell, makes provision of blood for patients who have developed antibodies against them, easier and less time consuming [6]. Prophylactic matching for Rh and Kell antigens for transfusions have been limited to multiply transfused population [3] and little is known for

the accidentally transfused or routine general hospital patients.

Rate of Alloimmunization

The rate of alloimmunization is much higher in the multiply transfused patients with a frequency of 3–50 % reported worldwide depending on the homogeneity of the donor and recipient population, RBC phenotype matching policy and age at transfusion [3, 7–9]. Indian studies quote an incidence rate of 3.4–9.5 % [9–12].

The alloimmunization frequency in general hospital patients was reported to be 1.35 % in Denmark [13], 0.78 % in Germany [14], and 0.3–2 % in the USA [15, 16]. Ko et al. [17] screened 22,463 cases for alloantibodies over a period of 2 years in surgical patients of which, 340 (1.52 %) cases had alloantibodies detected in them. In a 20 year retrospective study by Schonewille et al. [18] on 653 accidentally transfused population, alloimmunization was described between less than 1 to 10 %.

Out of the 500 patients studied in the present study, only one patient developed an alloantibody after 4 weeks after transfusion. The rate of alloimmunization in the study patients was 0.2 % as compared to 0.46–3 % reported in few studies on non chronically transfused or in general hospital patients [17, 19].

Several factors might have contributed to the lower rate in the present study. Firstly, antibody screening was done using the commercially available cell panels which are not indigenous. It could be different from the antigenic profile of our population and therefore, might have missed some antibody. [20].

Secondly, prestorage leucodepleted PRC were transfused which could have contributed to lower rate of alloimmunization. Singer et al. [7] observed that only one patient receiving prestorage leucodepleted blood developed alloantibody as compared to thirteen patients receiving non leucodepleted blood and five patients receiving post storage leucodepleted units.

Thirdly, we detected only clinically significant antibodies, which are predominantly IgG in nature. The higher alloimmunization risk in other studies could be attributed to identification of both IgM and IgG antibodies [17].

In a study by Alves et al. [2] of 143 individuals studied, 15 (10.49 %) developed alloantibodies which was higher in comparison to our results. The inclusion of 30 previously transfused patients into their study population might have led to a bias, as the developed alloantibody could be due to a secondary immune response. Also, the follow-up period was for 6 months, while we limited it till 4 weeks. As alloantibodies may take longer duration to develop, following up our patients beyond 4 weeks could have helped further.

As paediatric population was not included; we could not establish any relationship between alloimmunization and the age at which transfusion was initiated as against other studies. [21, 22] However, contrary to the notion that the immune system weakens at extremes of age thereby leading to immune tolerance [23], we had a patient who developed alloantibody beyond the age of 60.

The risk of alloimmunization to red cell antibodies varies from 0.77 to 1 % per unit transfused [10, 24, 25]. We noted a lower risk of 0.09 %, probably because our study population comprised only of cardiac surgery patients who are not multiply transfused. It could also be attributed to the lower number of mean units transfused in the present study, which was 2.3 as compared to a mean of 8.5–47 units in other studies [9, 26–28].

Comparison of Alloimmunization Rates

Of the 250 patients who received randomly matched blood units, 1 (0.8 %) developed an alloantibody as compared to none among patients who received Rh and Kell phenotype matched blood units. Such comparative studies evaluating the incidence of alloimmunization have been reported only in multi transfused patients [3, 7, 21].

Distribution of Rh (C, c, E, e) and Kell (K) Systems

It is important to know the frequencies of the various antigens when dealing with alloimmunized patients to predict the availability of blood units that lack the corresponding antigen(s). The prevalence of 'D', 'c', 'C', 'E', 'e', and 'K' antigens was 93.6, 58, 87, 20, 98 and 3.5 % respectively in a previous study at our centre by Makroo et al., ($N = 51,857$) [6]. In a similar study by Sharma et al. ($N = 1000$), the frequencies were 91.6, 58.3, 84, 25.6 and 78.5 % respectively [29]. Similar distribution was noted in the present study as well (Table 3).

Immunological Response to Antigenic Exposures

The sequence of antigenicity of the Rh and Kell antigens in decreasing order is $D > K > c > E > C > e$ [29]. The most common alloantibodies encountered in day to day practice are against Rh (D, C, c, E, e) and Kell antigens followed by other blood group antigens [7].

Among the Rh and Kell antigens, 'D' antigen is considered to be the most immunogenic. However, non-responders to 'D' antigen have been noted. [31]. To the best of our knowledge, such information from Indian scenario, on non-responders is lacking for the major antigens of the Rh and Kell system.

In our study, none of the patients in Group A received any antigenic stimuli whereas 119 (47.6 %) in group B

received stimulus for at least a single antigen ($n = 93$; 37.2 %) or more ($n = 26$; 10.4 %). The exposure for antigens 'C', 'c', 'E' and 'K' were 52, 22.8, 39.3 and 5.3 % respectively in this study. The antigen frequency for 'e' antigen in our study was 100 % so the exposure against it could not be seen.

Out of the 119 patients who received antigenic stimulus, only one (0.84 %) developed alloantibody. None of the other patients exposed to antigenic stimuli developed any alloantibody implying most of the patients were non responders (99.16 %). This observation further strengthens the notion that the development of alloimmunization not only depends upon frequency of antigenic stimuli but also on certain other factors [32].

We provided all the patients with red cell units matched for the 'D' antigen, therefore its antigenicity cannot be commented upon, in this study. Although 'K' seconds 'D' in antigenicity [30] and has been found to be the most frequent alloantibody [2, 19] in few previous studies, none of our 13 (5.3 %) patients exposed to 'K' antigen developed anti-K. This is in accordance to Schonewille's [33] observation that for developing an antibody against 'K' multiple transfusions are required. He also observed that the predominant antibody formed after 5 years of transfusion were anti-K and anti-Fy^a.

The 'c' antigen is considered to be highly immunogenic and closely follows the 'K' antigen [30]. In spite of this fact we observed that, none of the 22.8 % of the patients lacking 'c' antigen who were exposed at least once ($n = 10$ who were exposed once and $n = 13$ who were exposed more than once) developed anti-c.

Among E negative patients, 66 were exposed once to the stimulus and 17 of them multiple times. One of them developed anti-E. Majority of the studies on general hospital patients quote commonest antibody detected as anti-E. [20, 34, 35]. The antigenicity of 'e' antigen could not be commented upon as the frequency of this antigen in the study patients was 100 %.

It was observed that 52 % of the 'C' antigen negative patients had one or more exposures to it. However, none of them were alloimmunized. This is in keeping with the fact that among the five major Rh antigens, 'C' antigen is one of the least immunogenic [30].

Our study was not without limitations. Firstly, transfusion of other blood components were not considered for evaluation. Secondly, the follow up of these patients for a long term period is required to ascertain the development of alloimmunization. Genetic factors play a role in alloimmunization and is closely related to ethnicity. All our patient population was majorly restricted to North India territory. So, studying a larger sample size with patients of varied ethnicity would add up to our knowledge.

In conclusion, matching for Rh and Kell antigens for all transfusions in routine general hospital patients did not reach statistical significance. The resources and time can be reserved for providing the same in cases of multiply transfused patients. Nevertheless, this paves a way for further large scale evaluation on larger study population.

Compliance with Ethical Standards

Conflict of interest None.

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