Role of Red Blood Cells (RBCs) Alloimmunization in Multiple Transfusions

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ABSTRACT

Background & Objectives: Appropriate and regular red cell transfusion remains the main treatment of choice for a large number of patients with multiple transfusions.This study has been carried out to assess the prevalence of and to provide frequency and distribution patterns of various types of irregular red cell alloantibodies in chronic renal failure patients.

Materials and Method: 50 patients of Chronic renal failure were studied. The saline method, Albumin method, Indirect coombs', and Three cell panel test used for detection of red blood cell alloantibody. The variables studied were rate of red cell alloimmunization, type and specificity of RBCs alloantibodies and factors contributes to development of RBCs alloimmunization like age, gender, age at start of transfusion, number of packed cell received and ethnicity.

Results: Out 50 patients of Chronic renal failure, 5 patients(10%)developed red cell alloantibodies respectively. The red cell alloantibodies were against Rh, Kidd, Kell, Duffy, Lewis, MNS and P system. Results of this study(P value>0.05) indicate low frequency of RBCs alloimmunization. **Conclusion**: Low alloimmunization rate implies that there is homogeneity of red cell antigens in blood donors & recipients. RBCs alloantibody formation was not influenced by gender,age at start of transfusions and number of packed cells received**.** Already alloimmunized patients get benefits from leucodepleted packed cells. Specific recommendation given on routine pre-transfusion antibody screening to ensure safer transfusion.

Key words: Antibody screening, Chronic renal failure, Red cell alloimmunization,, leuco depleted packed cells.

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INTRODUCTION

Anemia is a severe complication of chronic renal disease that is common in more than 80%patients with impaired renal function[1].Although,there are many mechanism involved in pathogenesis of anemia (Iron, folate or vitamin deficiency, gastrointestinal bleeding, severe hyperparathyroidism ,systemic infection and shortened red blood cell survival),primary cause is inadequate production of erythropoietin by the damaged kidney. Prior to the introduction of erythropoietin,Hb levels of 5-7gm/dl were common in haemodialysis patients, who often required frequent blood transfusion when iron and anabolic steroid treatment failed to improve the clinical symptoms of anemia.

Despite the presence of hundreds of mismatched antigens between donor and recepients in every unit of RBCs, only 2-8%chronically transfused patients develop RBC alloantibodies.However,30-80%of Rh (D)negative patients who receive Rh(D)positive RBC units develop an anti-D antibody[2,3,4,5].The RBC alloimmunization rate following multiple transfusions in patients undergoing hemodialysis has been reported to be between about 6 and 10%, without a correlation between the number of RBC units transfused and alloantibody formation[6,7]. The antibodies detected mostly involved antigens in the Rhesus and Kell systems[6,7] .The presence of RBC alloantibodies may make finding compatible, antigen-negative RBC units difficult especially if multiple alloantibodies are found and may increase the risk of developing a DHTR. Several factors influence the rates of alloimmunization including antigenic differences, dose, frequency of transfusion, recipient immune/disease status and recipient HLA type[2].

OBJECTIVES

Appropriate and regular red cell transfusion remains the main treatment of choice for a large number of patients with multiple transfusions. Based on this the objectives of this study to assess the prevalence of red cell alloantibodies, to provide frequency and distribution patterns of various types of irregular red cell alloantibodies in chronic renal failure patients.

MATERIALS AND METHOD

Total 50 patients of CRF Medicine department, Sir T.General Hospital, Bhavnagar were included in study. Informed

consent was taken from all patients prior to collection of blood sample. All the details were collected in case record form. The study was started from 7th March 2014 to August 2014 on multiple transfused patients (50 cases) of CRF patients admitted in Sir T. General Hospital, Bhavnagar.

Antibody identification was carried out on serum employing by following method:

- ➤ Saline method
- ➤ Albumin method
- ➤ IAT
- ➤ Three cell panel

The variables noted were age, gender along with frequency and distribution of irregular red cell alloantibodies. The frequency of transfusion in patients who developed irregular red cell alloantibodies also noted.

STATISTICAL ANALYSIS

AGraphpad software was used for the statistical analysis. To analyse association between RBC alloantibody and age at start transfusion,gender and number of packed cell unit received, a non parametric method i.e.Fisher-exact was used.Two-tailed p value of <0.05(with Yates correction)were considered to indicate statistical significance.

OBSERVATION AND RESULTS

Table 1: Demographic data of chronic renal failure patients who received regular blood transfusion (N=50)

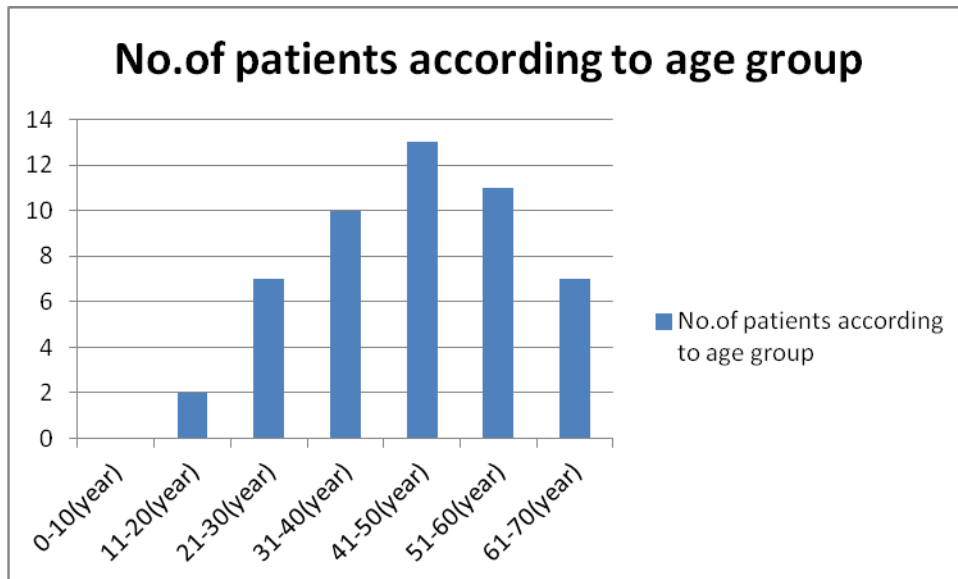| Diagnosis	Gender		Total
	Male	Female	
Chronic Renal Failure	36 (72%)	14 (28%)	50

Figure 1: Distribution of patients by age group

Figure 1.1: Number of packed cell transfused to patients
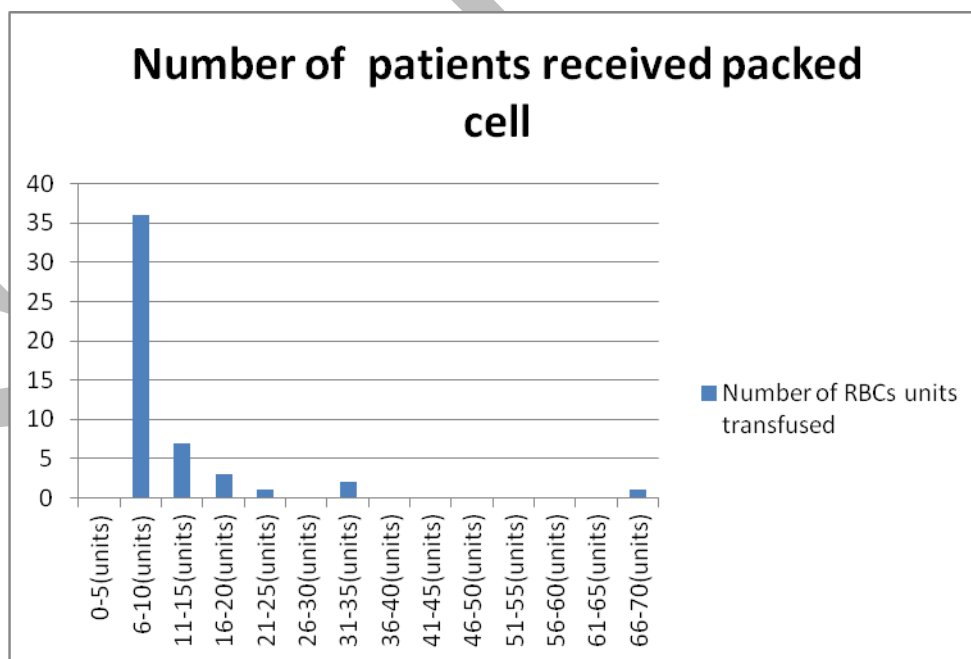
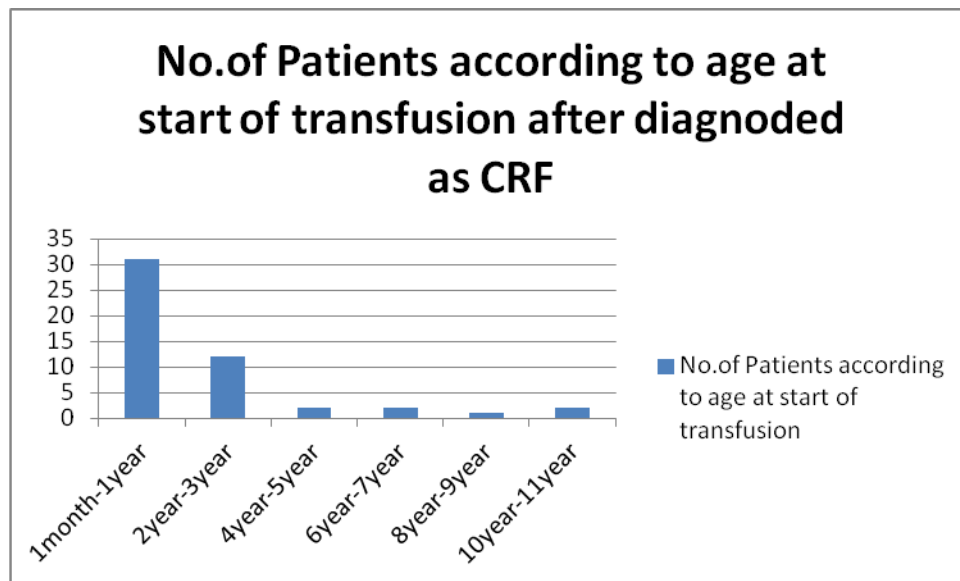Figure 1.2: Distribution of patients by age of start of transfusion after diagnosed as CRF

Figure 1.3: Specificity of method used for detection Of RBCs alloantibodies
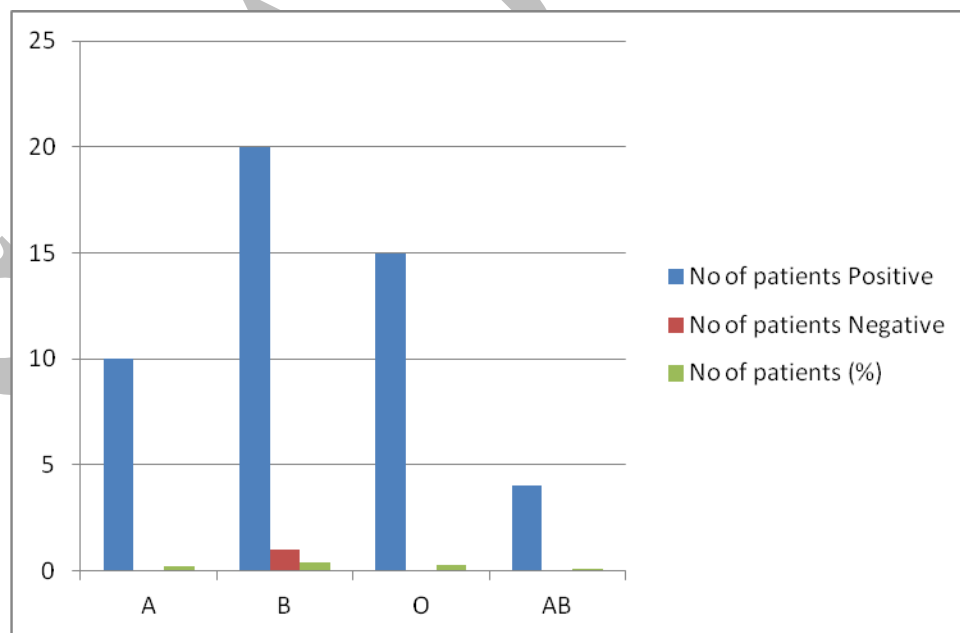
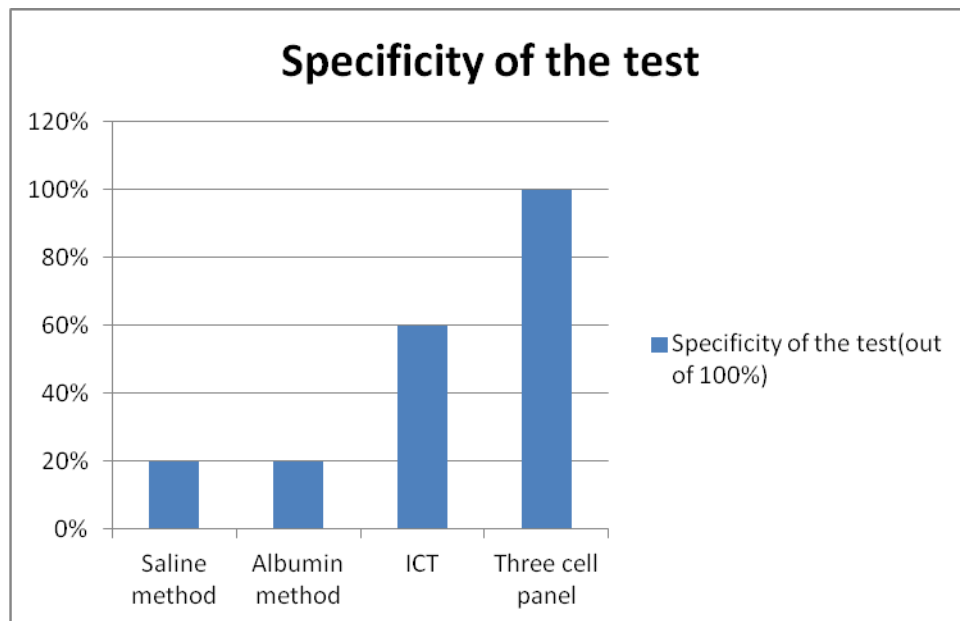Figure 1.4: Specificity of method used for detection of RBCs allo antibodies

Table 1.1: Association Between Alloantibody and Age at Start of Transfusion, Number of Packed Cell Transfused and gender of Chronic Renal Failure Patients were shown in Table (1.1)Below:

Independent variable	Present of alloantibody	Absent of alloantibody	P value(0.05 considered significant)
Age at start of transfusion after diagnosed as chronic renal failure			P value-1.000
<1year	4(8%)	32(64%)	
>1year	1(2%)	13(26%)	
Number of packed cell received			P value-1.000
<10units	4(8%)	32(64%)	
>10units	1(2%)	13(26%)	

Gender			
Male	5(10%)	31(64%)	P value-0.3041
Female	0(0%)	14(26%)	

Age at start of transfusion after diagnosed as CRF <1 year :Presence of alloantibody in 4 patients(8%) and absence of alloantibody in 32 patients(64%).Age at start of transfusion after diagnosed as CRF >year: Presence of alloantibody in 1 patient (2%) and absence of alloantibody in 13 patients(26%).This data showed P value 1.000 indicate there was no significant association between rate of RBCs alloimmunization and age at start of transfusion.

Number of packed cell received(<10 units): Presence of alloantibody in 4 patient(8%) and absence of alloantibody in 32(64%) patients. No of packed cell received(>10 units): Presence of

alloantibody in 1 patient(2%) and absence of alloantibody in 13 patients(26%).This data showed P value 1.000 indicate there was no significant association between rate of RBCs alloimmunizationand number of packed cell received.

Gender (Male): Presence of alloantibody in 5 patients(10%) and absence of alloantibody in 31(64%) patients. Gender (Female): Presence of alloantibody in 0 patient(0%) and absence of alloantibody in 14(26%) patients.This data showed P value 0.3041 indicate there was no significant association between rate of RBCs alloimmunization and gender.

DISCUSSION

The development of red cell antibodies occurs in a variable number of multiply transfused patients. In such circumstances, transfusion therapy may become significantly complicated. Effects of alloimmunization may include difficulty in finding compatible RBC units because of the presence of clinically significant RBC antibodies, transfusion reactions, or platelet refractoriness.[8] Present study is an effort to characterize blood group alloantibody formation in the patient population as only a few studies, mostly in non-Asian multiply transfused patients, have investigated the frequency and causes of red cell alloimmunization in the past.

Incidence of alloantibodies in multiply transfused chronic renal failure patients

In present study,red cell alloimmunization rate was found to be 10% in chronic renal failure.

Habibi and Lecolier[9] reported the incidence of red cell alloimmunization to be 1.72% in multi-transfused patients for hemodialysis.

In a study by Domen and Ramirez *et al*,[10] incidence of alloimmunization in chronic renal disease patients on hemodialysis was found to be 6.1%

Shukla and Chaudhary *et al*,[11] published a study where in they investigated the frequency of red cell alloimmunization in multi-transfused chronic renal failure patients undergoing hemodialysis. An alloimmunization rate of 9.8% was observed.

Previous studies reporting low rate of alloimmunization (5 to 10%) include those by Chaudhary *et al*,[11] Blumberg *et al*,[12] and Hmida*et al*.[13] A high rate of approximately 20% was noted in studies by Spanos*et al*, [14] Singer *et al*, [15] and Ameen*et al*.[16]

Patients′ age at the start of transfusion

It has been observed that an earlier start of transfusion may impart immune tolerance in some patients. [14,17] Our study showed no significant association between alloimmunization and age at start of transfusion.

Specificity of RBC alloantibodies detected

The specificity of most alloantibodies detected in our study was against Rh and Kell antigen systems due to their high immunogenicity, which is similar to previous reports of alloimmunization.[18]

The antibodies include Rheseus C,Rheseus c,Rheseus e,Kell K,Kell k, Duffy Fy[a], Kidd Jk[a], Ki ddJk[b],LewisLe[b], Lewis Le[a],MNS M,MNS N,MNS s, and P1.Hence, the transfusion of blood matched for Rh and K antigens could prevent alloimmunization resulting in a significant difference in the alloimmunization rates, but the potential to form RBC alloantibodies to unmatched antigens will exist. [19]

Gender and alloimmunization

In our study, all 5 alloimmunized patients were men, however gender was not a risk factor for alloimmunization. Females have been observed to be more prone to development of alloimmunization than males, [20] probably due to the fact that females, especially in developing countries, are anemic and pregnancy is an important risk factor for alloimmunization.

Effect of using leuco depleted blood

Majority of the patients in the our study had not received leucoreducedblood.

Another important aspect that has emerged is the role of contaminating leucocytes of the allogeneic blood transfusion in causing immunomodulatory effects in the recipient. Contaminating leucocytes down regulate T-helper cell type 1(Th1) immune response and drive the

recipient towards a T-helper cell type 2(Th2) responses. Such skewing towards type 2 immunity may enhance alloantibody formation. [21]Leucodepletion also removes donor APCs, abrogating the direct pathway of alloimmunization by donor-recipient T cell interaction. Donor leucocytes are known to readily express activation and co-stimulatory molecules upon recognition of recipient antigens.[22] Besides this, both autologous and allogeneic non-leucodepleted blood components release soluble bioactive mediators during storage which mediate some of the Transfusion Related Immunomodulation effects, and the Prestorage leucodepletion has been shown to prevent some deleterious effects. [23]

Number of transfusions received

In our study 4 patients developed alloantibody at or before 10 units and one patient developed alloantibody after 10 transfusion.The risk of developing alloimmunization was not very clearly associated with the number of transfusions received in our study. Some of the earlier studies have found a strong correlation between the number of blood units transfused and alloantibody formation[24,25]while other studies have found no relationship between the number of transfusions and alloimmunization rate. [26,27,28]

Monitoring of RBCs alloantibody after each transfusion episode

Monitoring of patients for RBC antibodies after transfusion and repeating this after each transfusion episode [29] i.e. 72 hours after the first transfusion ensures that the transitory antibodies are not missed.

Newer techniques of antibody identification

Antibody screening was performed using column agglutination technology with

the gel cards and solid phase red cell adherence technology. This increased the sensitivity of detection as antibodies present in low titres could also be detected as has been proven earlier.[30]

Applicability of local cell panels

The screening cells used for screening alloantibodies, presently, have to be procured from abroad which incurs high cost when used routinely. These cells have short shelf life, get damaged in transportation and are expensive when imported to meet the needs for typing and screening large patient population. These problems can be done away with by using indigenously prepared cell panels [31] or screen cells and panels manufactured by some Nationalized Blood Transfusion Centres could be another alternative.

An additional advantage of cells from the local ethnic groups would be a better detection of antibodies in local population

while imported cells may miss certain antibodies against antigens in local population.[31]There are certain antigens that are predominantly found in the Asian population. One such antigen is the Mi11 phenotype of the Miltenberger subsystem (or GP Mur), this antigen being relatively common in Southeast Asia, especially along the south-east coast of China and Taiwan. [32] There is a possibility that such antigens may also be present in the Indian population. Since the screening cells are made from donors of mainly Caucasian descent, they will be lacking such antigens and in that case the antibodies, if present, will not be picked up during the antibody screening. This may be a hindrance in implementing type and screen in countries such as India. In the Peoples′ Republic of China, they have included red cells expressing Mi(a) and Di(a) because of the relatively high

frequency of both these antibodies and antigens in their population.[33,34]

Provision of extended red cell phenotying and use of phenotype-matched blood units

The benefits of extended red cell phenotyping to minimize alloimmunization have been debated in literature [35] but cross matching for Rh and Kell systems, obtained after doing extended red cell phenotyping of patients and donors, from the time of initial transfusion, has been reported to lead to significant decrease in the alloimmunization incidence rate. Definitive advantages of RBC phenotyping include identification of the RBC antigenic profile among regular repeat donors for the ease of availability of compatible blood for multiply transfused patients.[35]

CONCLUSION

The incidence of alloimmunization depends on the demography and the population being studied. Such characteristics as age, sex , the type of hospital and the subpopulation being studied made it difficult to relate the experience of one institution to another.

Our data showed low alloimmunization rate in multiple transfused chronic renal failure patients. The factors that might contribute to this finding were the similarity of patients and donors ethnicity.

However, even though the immunization rate was low, we still recommend routine RBC antigen phenotyping for all multiple transfused chronic renal failure patients before starting RBC transfusion and providing pre storage leucodepleted blood matched for ABO and Rhesus antigen.

RBC phenotyping should also be performed on donors to identify the RBC antigenic profile.This would increase the avaibility of compatible blood for chronic renal failure patients.

Unless preventive measures applied,the rate of RBC alloimmunization would increase for these chronic renal failure patients,who had a long term need for RBC transfusions.Those who had developed an alloantibody should be given fully phenotypically matched blood in order to prevent further alloimmunization.

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