International Journal of Research in Medical Sciences
Patel AS et al. Int J Res Med Sci. 2016 Mar;4(3):822-828
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20160526

Research Article

Role of RBC's alloimmunization in multiple transfused thalassaemia patients

Amita Sagar Patel[1]*, Sejal Gamit[1], Mayuri Gohil[2]

[1]Department of Pathology, Govt. Medical College, Surat, Gujarat, India
[2]Department of Pathology, Govt. Medical College, Bhavnagar, Gujarat, India

Received: 13 January 2016
Revised: 18 January 2016
Accepted: 08 February 2016

***Correspondence:**
Dr. Amita Sagar Patel,
E-mail: amita1883@gmail.com

ABSTRACT

Background: 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 thalassaemia major patients.
Methods: 50 patients of thalassemia major 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 thalassemia major, 4 patients (8%) 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.
Conclusions: 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.

Keywords: Antibody screening, Thalassaemia, Red cell alloimmunization, Leucodepleted packed cells

INTRODUCTION

Thalassaemia is one of the major public health problems. It is a heterogeneous group of inherited autosomal recessive disorder of hemoglobin synthesis, which is characterized by the absence or reduced output of one or more globin chains of haemoglobin.[1]

Clinically thalassaemia are classified according to their severity into major, intermediate and minor forms. Thalassaemia major is a severe transfusion dependent disorder. Thalassaemia intermedia are characterized by anaemia and splenomegaly and require non-regular transfusion. Thalassaemia minor is the symptomless carrier state.[1]

Present management consist of regular monthly blood transfusions to maintain the mean haemoglobin level of 10-11gm/dl and this remains the main treatment for severe Thalassaemia.[1] One of the complications of blood transfusion is the formation by the recipients of alloantibodies and autoantibodies against red cell antigen. Results from a number of studies had demonstrated various frequencies and percentages of alloantibody and autoantibody formation in transfused patients. Some alloantibodies were haemolytic and might cause haemolytic transfusion reactions and limit the availability of safe transfusion, while others were clinically insignificant. Red cell autoantibodies appeared less frequent but could result in clinical haemolysis and difficulty in cross matching blood.[2]

Antibodies must systematically be identified in the recipient's serum before every transfusion so that compatible blood could be provided. Otherwise problems might occur which sometimes could even threaten the patient's life.[3] Prevention of alloimmunization ranged from provision of red blood cells matched for all major antigens associated with clinically significant antibodies to blood matched only for antibodies that had already been made. This was because the fact that many alloantibodies were not harmful. Although expensive prevention methods might benefit only some patients.[2]

Alloimmunization may exist and not be recognized because:

1. The serum is not examined at the appropriate time following antigenic challenge.
2. The antibody strength is below the threshold for detection.
3. The target cells do not possess the corresponding antigen.
4. The immunity is cellular not humoral.[4]

Clinically significant RBC alloantibody in multiple transfused patients can pose major problems in long term transfusion therapy. Traditionally the rate of alloimmunization was thought to increase with the total quantity of blood transfused found that RBC alloimmunization is most frequent during the first 15 transfusion.[5,6] They also suggested thalassaemia patients become alloimmunized to a particular antigen because of underlying capacity for immune response in the initial exposure. Study done by Spanos et al found that early onset of transfusion (age <3 years) is associated with lower frequency of alloantibody formation.[8] When antibodies against high frequency antigens develop, it can be very difficult to find suitable blood donors. Most blood transfusion services match only red cell units for ABO and Rh antigens.

Antigen matching transfusion would effectively prevent alloimmunization. To do so, the patient's ABO, Rhesus, Kell, Kidd and Duffy and Lewis systems should be typed at diagnosis or before institution of transfusion therapy. Blood to be transfused should always be matched at least with ABO, Rhesus and Kell system.[7]

Objectives

- To find out incidence of various RBC alloantibodies as well as autoantibodies in repeatedly transfused patients.
- To determine the type of antibody present in multiple transfused patients.
- To identify the factors such as frequency of transfusion, splenectomy status, donor ethnicity and gender and their association with the development of antibody in repeatedly transfused patients.
- To identify common RBC alloantibodies.

So, the aim and objectives of this descriptive study is to assess the prevalence of and to provide frequency and distribution patterns of various types of irregular red cell alloantibodies in patients with thalassaemia major.

METHODS

50 patients of thalassaemia major from pediatric department, Sir T. General Hospital, Bhavnagar were included in study from march-August (2014). Informed consent was taken from all patients prior to collection of blood sample. All the details were collected in case record form. Antibody identification was carried out on serum employing:

- 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

A Graphpad software was used for the statistical analysis. To analyze 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.

RESULTS

Demographic data of thalassaemia major patients who received regular blood transfusion (N=50) was given in (Table 1).

Table 1: Demographic data of thalassaemia major patients who received regular blood transfusion (N=50).

Demographic data	No. of patients	%
Total patients	50	
Diagnosis		
B Thalassaemia major	50	100%
Gender		
Male	29	58%
Female	21	42%

The data in (Table 2) show P value >0.05 indicate there was no significant association between rate of RBCs alloimmunization and age at start of transfusion, no significant association between rate of RBCs alloimmunization and number of packed cell received

and no significant association between rate of RBCs alloimmunization and gender.

Table 2: Association between alloantibody and age at start of transfusion, number of packed cell transfused and gender of thalassaemia major patients were shown in table below.

Independent variable	Present of alloantibody	Absent of alloantibody	P value (<0.05 considered significant)
Age at start of transfusion			1.000
<1 year	4	37	
>1 year	0	9	
Number of packed cell received			0.2914
<10 units	1	3	
>10 units	3	43	
Sex			1.000
Male	2	19	
Female	2	27	

Table 3: Comparison of red blood cell alloimmunization rate in various studies.

Study	Rate of alloimmunization
Present study	8%
Ho et al[14]	7.4%
Sirchia et al[15] and Spanos et al[3]	Ranged from 5%-7%
NHM Noor[16]	7.9%
Michail et al[17]	23.43%
Singer et al[2]	22%

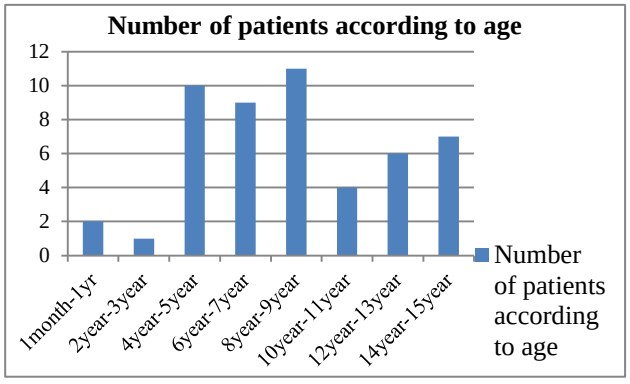

Figure 1: Distribution of patients by age group.

Distribution of patients by age group, Number of packed cell transfused to patients, Distribution of patients by age of start of transfusion after diagnosed as thalassaemia major, Specificity of method used for detection of RBCs allo antibodies were shown in Figure 1-4 respectively.

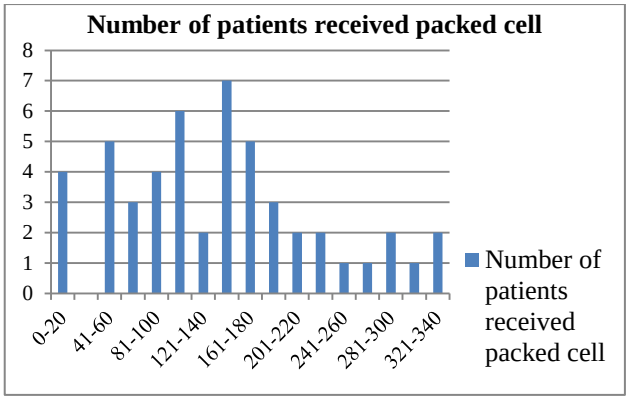

Figure 2: Number of packed cell transfused to patients.

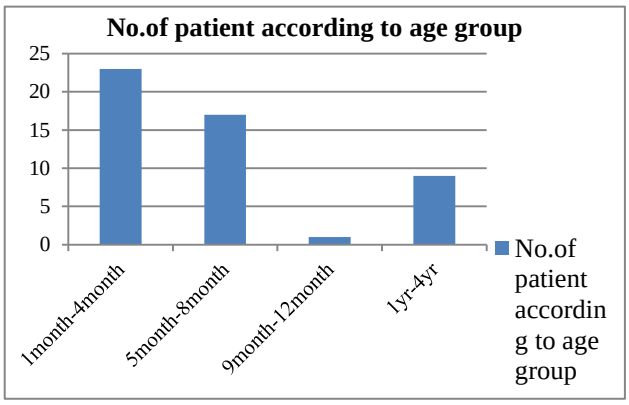

Figure 3: Distribution of patients by age of start of transfusion after diagnosed as thalassaemia major.

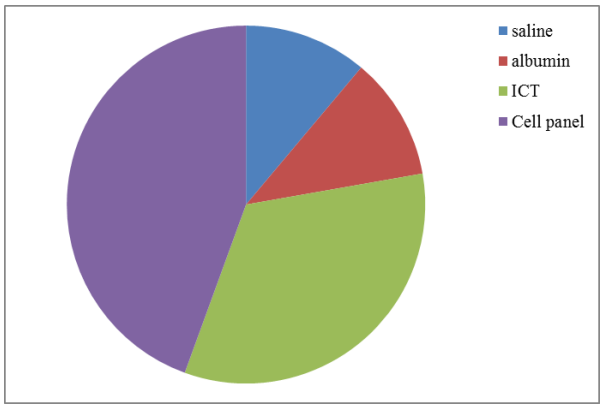

Figure 4: Specificity of method used for detection of RBCs alloantibodies.

DISCUSSION

Efficacy of transfusion regime

Thalassaemia is a major public health problem. This prospective study of alloimmunization to red cell antigens in multiple transfused thalassaemia patients was first carried out in HUSM and HKB. It was undertaken to estimate the proportion of red cell antibody in multiple

transfused thalassaemia patients and to find out factors contributing involved at least 3 main contributing elements; the RBC antigenic difference between the blood donor and the recipient; the recipient immune status and immunomodulatory effect of the allogenic blood transfusion.[9]

Patients presenting at the age 1 or 2 years of life were transfusion dependent for life.[10]

Majority of our patients started on transfusions between the ages of 3month to 1 year old. One patient started transfusion at the age of 1month, while one patient started transfusion at 3years old. These patients were diagnosed as beta thalassaemia major and initially required irregular transfusions, but now become transfusion dependent and required transfusions monthly to 2-3weekly respectively.

In study by Spanos et al majority of their patients were started on transfusions before the age of 3 years old.[3]

In Kuwait, all of patients studied were started on transfusions before the age of 12months old.

We practiced hypertransfusion regime in our hospital and the amount of blood to be transfused were based on the aimed haemoglobin. The cut off point for pre and post haemoglobin level were depending whether the patients were on iron chelator or not.

For thalassaemia major who were on iron chelator, the pre transfusion hemoglobin level was less than 10 gm/dl and post transfusion hemoglobin level was 14 gm/dl. Thalassaemia major who were not on iron chelator, the pre transfusion hemoglobin level was less than 10 gm/dl and post transfusion hemoglobin level was 12 gm/dl. Patients who were not on iron chelator usually due to serum Ferritin less than 1000 µg/l, unable to get desferrioxamine infusion pump because of socioeconomic problems, age less than 3 years old or newly diagnosed.

Sensitivity and specificity of laboratory methods

Alloimmunization may exist and not be recognized because 1) the serum is not examine at appropriate time following antigenic challenge; 2) the antibody strength is below the threshold for detection; 3) the target cells do not possess the corresponding antigen: 4) serologic test are not optimal; 5) the immunity is cellular, not humoral.[4]

In our study, to detect the presence of IgG antibodies several media (22% bovine albumin) used to enhance and potentiate the antigen-antibody reaction in Bovine albumin method and antihuman globulin sera used in ICT and Three cell panel method to detect IgG.

Several reagents are aimed at reducing the net negative charge (zeta potential) on the surface of red cell. The other regents used for this purpose are enzyme, LISS. LISS and ID-Diluent 2 were reported to improve red cell antibody detection in Diamed –ID LISS/Coombs Gel cards following incubation at 37C for 15 minutes (Walker RH et al).[11]

A combination of factors influences antibody-antigen binding and the performance of gel card detection systems. Skill and practice are required to detect the difference between a negative and a very weak reaction, especially when carried out by non dedicated transfusion laboratory staff. (Pahuja et al).[12]

Frequency of ABO blood group system

We observed that 48 patients in this study were Rhesus positive and 2 patients were Rhesus negative. Majority of patients were blood group B (38%), while other were group O (32%), group A (22%) and group AB (8%). There was no difference in the proportion of blood group between our patients and local data on donor's blood groups, which showed that were group B (40%), group O (36%), group A (19%) and group AB (5%). Majority of our donor were Rhesus positive which comprise about 99% and Rhesus negative in 1% of donor (unpublished data). A same blood group genotype can minimize problems in antibody screening and identification of testing as well as in the supply of compatible blood.

Frequency of minor antigen system

With regard to Rhesus system Rhesus C, Rhesus c and Rhesus e were common. With regard to Kidd system both JKa and JKb were common. In lewis system Lea was most common and in P system P1was most common. With regard to MNS system, there were high frequency of M antigen and low frequency for N and S antigen. Fya and Fyb both were common in study. Regarding Kell system, kk and kpa positive are 100%, in our study the incidence of Kell k is high, (Nathalang et al).[13]

In our study antisera D was used for identification of Rh negative recipient

Other than the A and B antigens, Rh (D) is the most important red cell antigen in transfusion practice. In contrast to A and B, however, persons whose red cells lack the Rh (D) antigen do not regularly have the corresponding antibody. Formation of anti-D almost always results from exposure, through transfusion to red cells possessing the Rh (D) antigen. The Rh (D) antigens have greater immunogenicity than virtually all other red cell antigens except A and B antigens. More than 80%of Rh (D) negative persons who receive an Rh (D) positive blood transfusion are expected to develop anti-D antibodies. To prevent this, the blood of all recipients and all donors is routinely tested for Rh (D) to ensure that Rh (D) negative recipients are identified and given Rh (D) negative blood.

Type and specificity of red cell antibody

In this study, all of our patients received compatible blood for only ABO and Rh D antigen. Sirchia et al reported that the alloantibody found in their study was almost entirely confirmed to the common antigen of Rhesus, Kell, Kidd and Duffy system.[15]

Our study with consistent with this study because 4 patients who developed alloantibodies had antibody against Rheseus D, Rheseus C, Rheseus E, Rheseus c,Rheseus e, Kell k, Kell K, Duffy FYa, Duffy Fyb, Kidd Jka, Ki dd, Jkb, Lewis Lea, Lewis Leb, MNS M, MNS N, MNSs, P1system.

Factors contribute to the development of alloantibody

Our results showed that there was no significant association between alloimmunization and gender, as we observed that in our study 2 male and 2 female patients developed alloantibodies. Further study on bigger sample size was needed to exclude the influence of sex on red cell immunization.

Clinically significant alloantibodies had been reported to occur about twice as often in women compared with men even when women without previous pregnancy were compared with men who received blood transfusions.[4]

No difference in gender was found by other investigators.[2]

Of the 4 alloimmunized patients in our study, one patient was 4 month old, 2 patients were 12 years old and one patient was 4 year. There was no association between alloimmunization and age demonstrated in this study. Fluit et al, Singer et al and Ho et al reported that no significance relationship between age and alloimmunization in transfusion dependent thalassaemia patients.[2,14,18]

Our low alloimmunization rate in this study can also be explained by the similarity in the ethnic between patient and donor. All of our alloimmunized patients were Indians and all of them received blood donated by Indians. In Hong Kong majority of immunized patients were southern Chinese and all blood donors were predominantly of the same ethnic group. Lower rate of alloimmunization in their study was explained by their access to phenotypic ally matched donors in Hong Kong.[14] A study done by Dr Noor Haslani Mohd Noor in Malay thalassaemia patients showed lower alloimmunization rate as most of their blood donors were also Malays.[16] Our study was consistent with these two studies.

However, in Kuwait, the patient's ethnic background was classified as Kuwaiti Arab and non Kuwaiti Arab. The percentage of the Arab Kuwaiti donors ranged from 40% to43% and 42%to 45% of the donors were other Arabs, whereas non Arab Asian donors comprise 18% to 20% of all blood donors. A higher RBC alloimmunization among their patients were due to the heterogeneity of the population in Kuwait that influenced on the donor population. As a result of this heterogeneity, the expense and feasibility of providing extended antigen matched blood may not be possible each time.[19]

Study by Sirchia et al found lower red cell alloimmunization in patient who was transfused blood matched for ABO, Rhesus and K antigen. In Greece, a high alloimmunization rate of 22% overall and 20.8% among Asian patients.[15] This was because majority of the blood donors was white and the majority of blood donor recipients were Asians, so their erythrocytes antigen distribution also differed significantly.[2]

Effect of age at which transfusion start

We observed that all 4 of our immunized patients were started on transfusion before the age of 1 year old. However, our results showed there was no statistically significant association between rate and age at start of transfusion. This was most probably due to our small number of patients.

Ameen et al found majority of alloimmunised patients formed first antibody between the aged of 2 and 10 years (58%). They observed that most of the younger age.[19]

Michail et al reported that low frequency of alloimmunization found in patients with thalassaemia major who started transfusion treatment before they are 1 year old.[17] Their result also supported the view that there was some form of immune tolerance (induced by immature immune response mechanism to repeated blood transfusion).

Study by Spanos et al reported that early onset of transfusion (age <3 year old) was associated with a lower frequency of alloantibody formation. The resistance to alloimmunization was perhaps the consequence of immaturity of the immunological system, particularly antibody production.

Transfusion at an early age (<1-3 year old) might offer some immune tolerance and protection against alloimmunization in thalassaemia patients.[2]

Despite exposer to many RBC and WBC antigen, no infant produced alloantibody against blood cells antigen. The immunological mediated transfusion reactions should be quite rare in young infants.[18] Contrast to this study; in our study one infant patient had developed alloantibody.

Effect of number of packed cell received

In this study we found that number of packed cell that had been transfused ranged between 2 units to 336 unit

with mean of 47.1 units. We also observed that all the alloimmunized patients developed antibody after100 units, 40 units, 1 unit and 200 units respectively. Our results showed there were no significant relation between number packed transfused and alloimmunization rate.

Spanos et al in their study found that the earliest sensitization appear more or less 10 units transfusions.[3] Blumberg et al conclude that most blood group antibody seen in multiple transfused patients were due to previous pregnancy and to the initial 1-10 transfusion.[6] They also found that the rate of antibody formation per transfusion actually decreased with increasing number of transfusions.

However our result consistent with the findings by Fluit et al who showed that 36% of immunized patients developed alloantibody after many transfusions.[18] They conclude that the incidence of antibody formation increases with number of transfusions. This study was also supported by Singer et al who observed that immune response might be affected by number of blood units patients received.[2]

However the relation between the number of blood units transfused and antibody formation is still unknown in thalassaemia, but it was an important factor for increased alloimmunization in patients who received multiple transfusions.

Role of leukodepletion blood

The role of leukodepletion in prevent in alloimmunization was mentioned in a number of studies. It had been shown that storage of RBC at 1 to 6 ºC would induce apoptosis in WBCs.[20] The high percentage of apoptotic features of residual WBCs and loss of viability was shown at day 3 after storage. This might sensitize the immune system of the recipient and lead to the development of autoimmune disease.[21] All of the patients involved in this study had in term exposure to non leukodepleted and post storage blood. Therefore the potential donor's WBCs could influence on the rate of alloimmunization in our multiple transfused thalassaemia patients. In our study majority of the patients received packed cell of 2-7 day old. Singer et al observed that senescent erythrocytes would have conformational changes and might expose new antigens and promote or enhance immune reaction.

Our data showed low alloimmunization rate in multiple transfused thalassaemia 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 thalassaemia patients before starting RBC transfusion and providing prestorage 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 availability of compatible blood for thalassaemia patients.

Unless preventive measures applied, the rate of RBC alloimmunization would increase for these thalassaemia patients, who had a lifelong need for RBC transfusions. Those who had developed an alloantibody should be given fully phenotypically matched blood in order to prevent further 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 numbers of packed cells receive. Specific recommendation given on routine pre-transfusion antibody screening to ensure safer transfusion. To anticipate future developments, such as large-scale extended phenotyping of recipients and donors, the kinetics and frequency of RBC alloimmunization need to be more precisely determined through prospective studies in which antibody detection tests are performed at set time intervals after transfusion.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. George E. Thalassaemia carrier diagnosis in Malaysia. Thalassaemia diagnosis services (ThalIDS). 1998.
2. Singer St, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky E. Alloimmunization and erythrocyte autoimmunization in transfusion dependant thalassaemia patients of predominantly Asian descent.Blood. 2000;96:3369-73.
3. Spanos T, Karageorga M, Iadis V, Peristeri J, Hatziliami A, Kattamis C. Red cell alloantibodies in patients with thalassaemia.Vox sang. 1990;58:50-5.
4. Walker RH, Dong-tsamn Lin, Mary B. Arch pathol lab Med. 1989;113:254-60.
5. Brantly SG, Ramsey G. Red cell alloimmunization in multitransfused HLA-typed patients. Trasfusion. 1988;24:463-6.
6. Blumberg N, Ross K, Avila E, Peck K. Should chronic transfusion be matched for antigen other than ABO and Rh D Vox Sang. 1984;47:205-8.
7. Hoffbrand AV. Genetic disorder of haemoglobin. In post Graduate Haematology. Oxford University Press Inc, New York. 1999;91-119.

8. Spanos T, Karageorga M, I adis V, Peristeri J, Hatziliami A, Kattamis C. Red cell alloantibodies in patients with thalassaemia.Vox sang. 1994;58:50-5.
9. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. Blood. 2000;96:3369-73.
10. Castellino SM, Combs MR, Zimmerman SA, Issitt PD, Ware RE. Erythrocyte autoantibodies in paediatric patients with sickle cell disease receiving transfusion therapy: frequency, characteristics and significance. Br J Haematol. 1999;104:189-94.
11. Walker RH, Lin DT, Hartrick MB. Alloimmunization following blood transfusion. Arch Pathol Lab Med. 1989;113:254-61.
12. Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multitransfused thalassemics of Indian origin. Hematology. 2010;15:174-7.
13. Chuansumrit A, Nathalang O, Wangruangsathit S. HLA alloimmunization in patients receiving multitransfusions of red blood cells. Southeast asian j trop med public health. 2001;32:419-24.
14. Ho HK, Ha SY, Lam CK, Chan GC. Alloimmunization in Hong Kong southern Chinese transfusion-dependent thalassemia patients. Blood. 2001;97:3999-4000.
15. Sirchia G, Zanella A, Parravicini A, Morelati F, Rebulla P, Masera G. Red cell alloantibodies in thalassemia major. Results of an Italian cooperative study. Transfusion. 1985;25:110-2.
16. Noor Haslina MN, Ariffin N, Illuni HI, Rosline H. Red cell autoantibodies among thalassaemia patients in Hospital Universiti Sains Malaysia. Singapore Med J. 2007;48:922-5.
17. Michail-Merianou V, Pamphili-Panousopoulou L, Piperi-Lowes L, Pelegrinis E, Karaklis A. Alloimmunization to red cell antigens in thalassemia: comparative study of usual versus better-match transfusion programmes. Vox Sang. 1987;52:95-8.
18. Fluit CR, Kunst VA, Drenthe-Schonk AM. Incidence of red cell antibodies after multiple blood transfusions. Transfusion. 1990;30:532-5.
19. Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A. RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion. 2003;43:1604-10.
20. Frabetti F, Musiani D, Marini M. White cell apoptosis in packed red cells. Transfusion. 1998;38:1082-9.
21. Pistillo MP, Tazzari PL, Gaudiano C, Cilla V, Kato T, Matsui T, et al. Patients with neoplastic and nonneoplastic hematological disease acquire CTLA-4 antibodies after blood transfusion. Transfusion. 2001;41:462-9.

Cite this article as: Patel AS, Gamit S, Gohil M. Role of RBC's alloimmunization in multiple transfused thalassaemia patients. Int J Res Med Sci 2016;4:822-8.