Red Cell Alloantibodies in Multiple Transfused Thalassaemia Patients

Surg Cdr CN Chaudhari*

Abstract

Background: Thalassaemia major patients require lifelong transfusion support due to which they are prone for alloimmunization to foreign RBCs. Alloimmunization can be prevented by extended phenotype match blood transfusion. The study was conducted to know the extent of problem of alloimmunization and to find important red cell antibodies in thalassaemia patients.

Methods: A cross-sectional study was conducted. A total of 32 thalassaemia patients were enrolled. The specimen was subjected to red cell alloantibody and autoantibody by column gel agglutination technique. $R_1^wR_1$, R_2R_2 , rr (papaine and non papain) and 11 cell panel reagent cells were used in screening and identification of alloantibodies respectively.

Result: Six (18.8%) subjects were alloimmunized. All alloimmunized subjects were recipient of more than 20 units of transfusion. Total seven clinically significant alloantibodies were identified. Anti E and anti c were commonest antibodies in four (12.5%) patients.

Conclusion: Red cell alloimmunization is an important risk in thalassaemia patient. 71.4% of alloantibodies were anti E and anti c type. Extended phenotype match blood transfusion for Rh-c and Rh-E antigens or level 2 antigen matching stringency needs to be explored in preventing alloimmunization in thalassaemia patients.

MJAFI 2011; 67 : 34-37

Key Words : Alloimmunization; Irregular antibodies; Red cell alloantibodies; Thalassaemia

Introduction

Thalassaemia is associated with genetically determined reduction in rate of synthesis of one or more types of normal haemoglobin (Hb) polypeptide chain. The lack of polypeptide chain results in interference in erythroid maturation, function and ineffective erythropoiesis. β thalassaemia major is characterized by major or total suppression of β (Beta) chain synthesis in the homozygous form of the disease. Lifelong red blood cell (RBC) transfusion is the treatment of thalassaemia major patients which alleviates the anaemia and suppress the compensatory mechanism responsible for clinical disease including deaths in these patients. Bone marrow or stem cell transplantation is the other modality of treatment of thalassaemia, which is out of reach for most of them. Thus RBC transfusion is only treatment available to these patients [1].

Alloimmunization i.e. development of alloantibody against the foreign RBC is one of the important complications of blood transfusions in multiple transfused thalassaemia patients [2,3]. Alloimmunization further complicates the transfusion therapy due to difficulty in getting compatible blood, increased incidence of

additional alloantibody and autoantibody (antibody against self RBC antigens) development, delayed haemolytic transfusion reaction (DHTR) and life-threatening hyperhaemolysis syndrome [2-5]. Alloimmunization rate of 5-30% has been reported in thalassaemia patients by various workers [5]. Extended phenotype matched, leucodepleted red cell transfusion is recommended in prevention of alloimmunization [4,6]. There are large numbers of β thalassaemia major patients in India; however, data of alloimmunization is very sketchy. In this background the study was undertaken to find out the prevalence of alloimmunization in thalassaemia patients and to know important alloantibodies in alloimmunization.

Material and Methods

A cross sectional study was undertaken. Patients of thalassaemia major on regular transfusion were enrolled. Information on transfusion history of these patients was recorded. Five ml blood was collected from each subject and plasma or serum was separated. $R_1^wR_1$, R_2R_2 , rr (papaine and non papain) 'screening cell' were used as reagent cells for screening irregular antibodies, which was undertaken by column gel agglutination (CGA) technique at 37°C and room temperature (23°C) using LISS Coombs and NaCl gel cards

*Professor, Department of Microbiology, AFMC, Pune-40.

Received : 05.09.2008; Accepted : 19.09.2010 E-mail : ceanchau@hotmail.com

(Di Med) respectively. They were also subjected to Direct Coombs test by CGA technique. Specimens found positive for irregular antibody were subjected to alloantibody identification using 11 cells panel by CGA. Panel cells have the known antigens consisting of antigens as- Rh-hr (D,C,E,c,e,C^w), Kell (K, k, Kp^a, Kp^b, Js^a, Js^b), Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), Lewis (Le^a, Le^b), P₁, MNS (M,N,S,s), Luth (Lu^a, Lu^b), Xg^a, Bg^{a+}. Results were interpreted based on cross-out method. Prevalence of alloimmunized with 95% Confidence Interval (CI) was calculated. Chi square test or Fisher exact tests were used to test the association between sex and alloimmunized status. Student's unpaired 't' test was used to compare age, age at first transfusion and duration from first transfusion with respect to alloimmunized status.

Results

A total of 32 β thalassemia major subjects were enrolled for the study. They were regular recipients of blood transfusion at interval of 3-5 weeks. They received ABO Rh D match homologous, non leucodepleted whole blood or packed RBC. None of them underwent splenectomy. Six subjects were found positive for RBC alloantibodies; none was positive for autoantibody. Thus the prevalence of alloimmunized was 18.8% with 95% CI 5.3-32.3%. A total of 22 (68.75%) study subjects were male while 10 (31.25%) were female, amongst them 18.2% (4/22) male and 20% (2/10) female were alloimmunized. There was no significant association between sex and alloimmunized status (Fisher exact two tail p value >0.05). The age of study subjects ranged from 1 to 18 years. Mean age of alloimmunized subjects was 12.0 ± 3.8 year as against 8.2 ± 3.4 year in non alloimmunized; the difference in mean was statistically not significant by Student's 't' test ($p > 0.05$, NS). Age distribution of

thalassaemia subjects is presented in Table 1. Mean age at first transfusion of study subjects was 2.7 ± 1.3 years; which was 2.6 ± 1.3 and 3 ± 0.9 year in non-alloimmunized and alloimmunized subjects respectively ($p > 0.10$, NS). Mean duration from first transfusion (transfusion dependence) in alloimmunized and non-alloimmunized thalassaemia patients was 8.5 ± 4.0 and 5.6 ± 2.5 years respectively, the same was statistically not significant by Student's 't' test ($p > 0.5$, NS). ABO blood group distribution revealed that 19 (59.4%), 6 (18.8%), 4 (12.5%) and 2 (6.3%) subjects were of O, B, A and AB blood group respectively; amongst them 4 (21.1%), 1 (16.7%), 1 (25%) subjects of O, B and A blood group respectively were alloimmunized. Only two subjects were Rh D negative and none were alloimmunized. The transfusion history revealed that 29 patients received more than 20 units of transfusion, one patient received 2 units while two received 15-19 units. All alloimmunized patients received more than 20 units of blood transfusion.

A total of seven alloantibodies were detected in six patients i.e. five subjects with one while one subject with two alloantibodies. Anti E followed by anti c with the frequency of three (42.9%) and two (28.6%) respectively were the most prevalent alloantibodies, however, anti Le^b and anti Jk^b was found in one (14.3%) subject each. One subject had both anti E and anti c antibodies. Profile of alloimmunized thalassaemia patients is given in Table 2.

Discussion

We reported 18.8% alloimmunization prevalence in thalassaemia patients. High alloimmunization in thalassaemia patient was reported from Taiwan (37%) [7], Arab (30%) [8], and Asian descent (22%) [9]. Compared to this lower alloimmunization was reported in thalassemia patients from Iran (5.3%) [10], Pakistan (9.2%) [11], (6.8%) [12] and Malaysia (8.6%) [13]. Very few studies from India reported alloimmunization in multiple transfused patients including thalassaemia patients. A study from North India reported alloimmunization rate of 3.4% in multiple transfused patients; they screened 531 patients [14]. Shukla et al [15] from Lucknow reported RBC alloimmunization rate of 9.8% in chronic renal failure patients undergoing haemodialysis. The differences in alloimmunization were attributed to at least three contributing factors: the RBC antigenic difference between the blood donor and the recipient, the recipient's immune status and the

Table 1

Age distribution of thalassemia patients

Age group (year)	Non-ALLO* (%)	ALLO* (%)	Total studied (%)
0-4	5 (15.6%)	-	5 (15.6%)
5-9	13 (40.6%)	2 (6.3%)	15 (46.9%)
10-14	8 (25%)	3 (9.4%)	11 (34.4%)
15-19	0	1 (3.1%)	1 (3.2%)
Total	26 (81.2%)	6 (18.8%)	32 (100%)

* ALLO-alloimmunized

Table 2

Profile of alloimmunized thalassaemia patients

Sex	Age (year)	Blood group	Units tx	Age at first tx years	Duration of tx years	Alloantibody specificity
F	18	O+	>20	4	14	Anti Le ^b
F	14	A+	>20	4	10	Anti c
M	8	O+	>20	2	4	Anti c, Anti E
M	8	B+	>20	3	4	Anti JK ^b
M	13	O+	>20	2	11	Anti E
M	11	O+	>20	3	8	Anti E

tx:- Transfusion

Table 3

Antigen matching stringency levels [23]

Level	Antigen set	Antigen number
1	AB (O), Plus; {Ag-neg}	3+ Ag Neg*
2	Level 1 Plus: c, C, e, E; K	+5
3	Level 2 Plus: Fy ^a , Fy ^b , Jk ^a , Jk ^b ; S, s	+6
4	Level 3 Plus: k; M, N; Do ^a , Do ^b , Hy, Jo ^a ; Lu ^a , Lu ^b	+9

* Ag -Neg- of corresponding alloantibody (ies) identified.

immunomodulatory effect of the allogenic blood transfusion on the recipients immune system [3,9]. Low rate of alloimmunization is expected in a population where there is homogeneity of RBC antigen between the blood donor and recipients [9]; when transfusion of extended phenotype blood group matched blood units [4,16] and leucodepleted blood [6] is being practised. The study subjects were transfused neither extended phenotype matched nor leucodepleted blood.

All alloimmunized subjects received more than 20 units of blood. Mean duration from first transfusion was 8.5 ± 4.0 and 5.6 ± 2.5 years (though statistically NS) in alloimmunized and non-alloimmunized subjects respectively; this is indicative of higher RBC exposure in alloimmunized patients. It has been reported that early age at transfusion may offer some immune tolerance and protect against alloimmunization [9]; we were not able to establish it as a risk factor for alloimmunization. Absence of spleen is also considered as one of the risk factor for alloimmunization [9], however, none of our patients had undergone splenectomy.

The study revealed anti E and anti c antibodies in 66.7% of alloimmunized subjects. Few studies from India reported antibody specificity due to alloimmunization [14,17,18]. Thakral B et al [14] reported anti-c (38.8%), being the most common specificity, followed by anti-E (22.2%), in multiple transfused patients from North India. The same group also published case reports of anti E and anti JK^b in thalassemia patients of Chandigarh [17] and anti c, anti E responsible for haemolytic disease of newborn [18]. Various authors also reported higher percentage of anti E and anti c in Asian population [9, 12,13]. Based on this, we hypothesise heterogeneous distribution of Rh-E and Rh-c antigens in our population; however, literature search revealed only two reports of Rh-hr profile from India [19,20]. Papiha [19] reported R₁R₁ ranging from 50% in north India to 70% in Mongoloid population in east India. Rh-hr phenotyping undertaken on 111 Rh-D positive subjects by National Institute of Biologicals, New Delhi [20] indicated Rh-c and Rh-E antigen frequency of 48.6% and 20.7% respectively. Further Rh-c and Rh-E are potent antigens with relative potency of 0.041 and 0.0338 respectively, next to the antigen potency of antigen Rh-D and K (Kell) [3]. This explains the higher anti c and anti E prevalence in the study populations and various studies from India; however, more extensive studies are needed to confirm the same. We had one subject who had both anti E and anti c alloantibody. Interestingly it has been reported that R₁R₁ subjects who developed anti E are prone for subsequent development of anti c and DHTR [19]. Anti c/E pair being the third commonest concurrent antibodies after anti K/E and anti D/C has been reported [22].

Extended phenotype matched blood can be one option in the prevention of alloimmunization [4,15,23]. It has been shown that the prevention of alloimmunization by transfusion of extensively matched blood will reduce the challenge of providing compatible blood in future [23]. Extended phenotype matched blood transfusion based on levels of antigen matching stringency and antigen match used by Klapper et al [23] is given in Table 3. In retrospect, if the study subjects would have been transfused extended phenotype matched blood for Rh-E and Rh-c antigen or level 2 antigen matching stringency [23] then possibly 66.7% (4/6) of alloimmunization or alloimmunization in four subjects (12.5%) could have been prevented. Based on the findings of the study and studies from India [14-20], there is a need for further investigation of Rh-c and Rh-E antigen matching or level 2 antigen matching stringency in prevention of alloimmunization [23].

To conclude, alloimmunization due to foreign RBC is an important adverse effect of blood transfusion in thalassaemic patients. The study revealed alloimmunization rate of 18.8% in them, anti E and anti c being the commonest alloantibodies responsible for alloimmunization. Extended phenotype matched blood transfusion for antigen Rh-E and Rh-c or antigen stringency level 2 need to be investigated to prevent alloimmunization in thalassaemia patients.

Conflicts of Interest

This study has been financed by research grants from the O/o DGAFMS, New Delhi.

References

1. Hoffbrand AV, Moss AH, Pettit JE. Essential Haematology. Essential Haematology 5th Edition Oxford: Blackwell Publishing 2005.
2. Schonewille H, Haak HL, van Zijl AM. Alloimmunization after blood transfusion in patients with hematologic and oncologic diseases. Transfusion 1999; 39: 763-71.
3. Eder AF, Chambers LA. Noninfectious Complications of Blood Transfusion. Arch Pathol Lab Med 2007; 131: 708-18.
4. Schonewille H, van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusion in a non hematologic alloimmunized patient cohort: is it time to take precautionary measures? Transfusion 2006; 46: 630-5.
5. Zumberg MS, Procter JL, Lottenberg R et al. Autoantibody formation in the alloimmunised red blood cell recipient and laboratory implications. Arch Intern Med 2001; 22: 285-90.
6. Blumberg N, Heal JM, Getting KF. WBC reduction of RBC transfusion is associated with decreased incidence of RBC alloimmunization. Transfusion 2003; 43: 945-52.
7. Wang LY, Liang DC, Liu HC et al. Alloimmunization among patients with transfusion-dependent thalassemia in Taiwan. Transfus Med 2006; 16: 200-3.
8. Ameen R, Al-Shemmari S, Al-Humood S et al. RBC alloimmunization and autoimmunization among transfusion-

- dependent Arab thalassemia patients. *Transfusion* 2003; 43: 1604-10.
9. Singer ST, Wu V, Mignacca R et al. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. *Blood* 2000; 96: 3369-73.
 10. Karimi M, Nikrooz P, Kashef S et al. RBC alloimmunization in blood transfusion-dependent beta-thalassemia patients in southern Iran. *Int J Lab Hematol* 2007; 29: 321-6.
 11. Bilwani F, Kakepoto GN, Adil SN et al. Frequency of irregular red cell alloantibodies in patients with thalassemia major: a bicenter study. *J Pak Med Assoc* 2005; 55: 563-5.
 12. Bhatti FA, Salamat N, Nadeem A et al. Red cell immunization in beta thalassaemia major. *J Coll Physicians Surg Pak* 2004; 14: 657-60.
 13. Noor Haslina MN, Ariffin N, Illuni Hayati I et al. Red cell immunization in multiply transfused Malay thalassemic patients. *Southeast Asian J Trop Med Public Health* 2006; 37: 1015-20.
 14. Thakral B, Saluja K, Sharma RR et al. Red cell alloimmunization in a transfused patients population: a study from a tertiary care hospital in north India. *Haematol* 2008; 13: 313-8.
 15. Shukla JS, Chaudhary RK. Red cell alloimmunization in multi-transfused chronic renal failure patients undergoing hemodialysis. *Indian J Pathol Microbiol* 1999; 42: 299-302.
 16. Castro O, Sandler SG, Houston-Yu P et al. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell diseases: theoretical and practical implications. *Transfusion* 2002; 42: 684-90.
 17. Thakral B, Saluja K, Sharma RR et al. Early onset multiple alloimmunization (anti-E and anti-Jk^b) in a thalassaemic. *Clin Lab Haematol* 2006; 28: 286-7.
 18. Thakral B, Agrawal SK, Dhawan HK et al. First report from India of haemolytic disease of newborn by anti-c and anti-E in Rh (D) positive mothers. *Hematology* 2007; 12: 377-80.
 19. Papiha SS. Genetic variation in India. *Hum Biol* 1996; 68: 607-28.
 20. National Institute of Biologicals (homepage on the Internet). Delhi: (updated 2008 February 11). Available from <http://nib.gov.in/index.htm>.
 21. Shirey RS, Edwards RE, Ness PM. The risk of alloimmunization to c (Rh4) in R1R1 patients who present with anti E. *Transfuion* 1994; 34: 756.
 22. Tormey CA, Stack G. The characterization and classification of concurrent blood group antibodies. *Transfusion* 2009; 49: 2709-18.
 23. Klapper E, Zhang Y, Figueroa P et al. Toward extended phenotype matching: a new operational paradigm for the transfusion service. *Transfusion* 2010; 50: 536-46.

ATTENTION ADVERTISERS

Rates of advertisements in Medical Journal Armed Forces India :

	Black & White	Colour	Size
Full page (per issue)	₹ 5000/-	₹ 10000/-	25 x 18 cms
Half page (per issue)	₹ 3000/-	₹ 6000/-	18 x 12 cms

(Positive for advertisement to be supplied by client)

Inserts (two pages)	₹ 5000/- per insert (Black and White / Colour)
	(Printed inserts to be supplied by client)

Address for Correspondence

EDITOR-IN-CHIEF

MEDICAL JOURNAL ARMED FORCES INDIA

C/O ARMED FORCES MEDICAL COLLEGE, PUNE - 411 040.