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Pathology

RED CELL ALLOIMMUNIZATION IN MULTIPLY TRANSFUSED THALASSAEMIA PATIENTS:A STUDY ON 300 CASES IN A TERTIARY CARE HOSPITAL OF NORTH EAST INDIA.

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ABSTRACT **Introduction & Background**: Alloimmunization i.e. development of alloantibody against the foreign RBC is one of the important complications of blood transfusions in multiply transfused thalassaemia patients.We studied the frequency of red blood cell(RBC) alloimmunization among thalassemia patients who received regular transfusions at our center and analyzed the factors, which may be responsible for development of these antibodies.

Patient/Material and methods: The study was carried out on 300 thalassemia patients in the age ranging from 2 to 40 years who were dependent on transfusion and had a history of blood transfusion at least once in every month. The patient's age, sex, ABO and Rh blood group, history of splenectomy, type of thalassemia,age of first transfusion and number of total blood transfusion were recorded.Alloantibody screening and identification was done using three cell and 11 cell panel respectively.

Results: Sixteen patients out of total 300 patients(5.33%) developed alloantibodies and 3 patients(1%) developed autoantibodies.A total of 19 alloantibodies were identified in patient population.The most common alloantibody was Rh-related (78.92%; 15 of 19), comprising anti-c (42.1%; 8 of 19),anti-E(26.3%; 5 of 19),anti-C & D (5.26% each; 1 of 19), followed by anti-Kell (10.5%; 2 of 19)comprising of anti-K,anti-Kidd (comprising of anti-Jka 5.26%; 1 of 19 and anti-Jkb 5.26%:1 of 19).

Conclusion : Alloimmunization was detected in 5.33% of multitransfused thalassemia patients. Rh and Kell blood group system antibodies accounted for more than 80% of alloantibodies.This study re-emphasizes the need for RBC antigen typing before first transfusion and issue of antigen matched blood (at least for Rh and Kell antigen).Early institution of transfusion therapy after diagnosis is another means of decreasing alloimmunization

KEYWORDS : Alloimmunization, Autoimmunization, Thalassaemia.

INTRODUCTION

The Thalassemias are a heterogeneous group of disorders caused by inherited mutations that decrease the synthesis of of either the α-globin or β-globin chains that compose adult haemoglobin, HbA (α2β2), leading to anemia,tissue hypoxia and red cell hemolysis related to the imbalance in globin chain synthesis.[1]Thalassemias are the major genetic disorders prevalent in certain parts of the world including india and hence are of great public health importance.[2,3] The β thalassemia is probably the most common inherited haemoglobin disorders in the Indian subcontinent and is the most severe form.The most severe forms of β-thalassemia major present within the first year of life with severe anaemia and failure to thrive[4] Study regarding the prevalence and incidence of beta-Thalassemia in India compared to that in the world and found that India has 3.3% prevalence of beta-Thalassemia and each year 10,000 babies with this disease are born in India which constitutes 10% of the total number in the world.

Life long 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[5]. 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.[6]Alloimmunization i.e. development of alloantibody against the foreign RBC is one of the important complications of blood transfusions in multiple transfused thalassaemia patients.[7]Development of alloantibodies against RBC antigens also complicate RBC cross matching,shortens in *vivo* survival of transfused cells,delays provision of safe transfusions and may accelerate tissue iron loading.[8]The risk of alloimmunization is more in thalassemia patients who are highly transfused and alloimmunization culminates in difficulty in obtaining the compatible blood[9].Because blood transfusion in these patients is necessary for life, early diagnosis and detection of these alloantibodies are very important.Early diagnosis by antibody screening is an important step in the detection of these alloantibodies.[10] In guidelines for chronic transfusions in patients with thalassemia,antigen phenotyping before the first blood transfusion, laboratory tests including CBC, cross-match and RBC antibody screening are recommended[11]. While antibody screening is included in the compatibility testing protocol in the developed countries, it is not yet available in India and other developing countries.[12,13] Reported alloimmunization rates ranged from 4% to 50% in thalassemia, and were lower in more homogenous populations. However limited data are available on the frequency of RBC alloimmunization in transfusion dependent thalassemia patients from north-eastern part of India, as pretransfusion antibody screening is not routinely performed. Thus this study was conducted in the Department of Pathology, Gauhati Medical College,Guwahati,Assam to determine the prevalence of RBC alloantibodies in transfusion dependent thalassemia patients,the types and specificities of these antibodies and to analyze the factors which may be responsible for development of alloantibodies.

MATERIALS AND METHODS:

The prospective and observational study was conducted in the Department of Pathology, Gauhati Medical College and Hospital for the duration of one year from August 2015 to July 2016. The study was conducted on 300 transfusion dependent thalassemia patients registered with thalassemia clinic at Gauhati medical college. Informed consent was obtained from patients or their parents.The study was approved by hospital ettics committee.

Thalassemia patients in the age ranging from 2 to 40 years who were dependent on transfusion and had a history of blood transfusion at least once in every month were evaluated in the study.The exclusion criteria were children with Non-Transfusion dependent Thalassemia and other Haemoglobinopathies as well as patients with background of HIV, HBV, HCV or any other infectious symptoms in recent two weeks. All the patients enrolled in the study interviewed using standard questionnaire to collect clinical data. The patient's age, sex, ABO and Rh blood group, symptoms, history of splenectomy,type of thalassemia(beta thalassemia major and E-beta thalassemia), age of first transfusion and number of total blood transfusion were recorded. Under aseptic conditions, A volume of 2mL blood is drawn into an

ethylene diamine tetraacetate (EDTA)containing tube, centrifuged at 3000×g for 3 minutes to obtain plasma (for antibody screening)and red cells (for detection of autoantibodies) on gel card system. Screening and identification of alloantibody was done by Diamed-ID column agglutination gel card technology using 3-cell and 11-cell panel respectively. Autocontrol was performed in each case to identify autoantibodies. It was done by incubating patient's cell with patient's plasma at 37^0 C for 15 minutes and then centrifuging for 10 minutes on gel card containing polyspecific antihuman globulin (anti-IgG + C3d). All the tests were performed using the gel card method by Diamed ID (Switzerland), as per manufacturer's guidelines.Finally according to presented antigram pattern of each panel, type of specific antibody against each antigen was determined.

Statistical analysis

Analysis was performed through SPSS software by making the frequency distribution tables and identifying frequency of alloimmunization and autoimmunization as well as the specificity of the particular alloantibodies.Discrete categorical data were presented as (%).Comparisons for categorical data were made by Chi-square test. All reported values are two-sided, with a significance level of 0.05.

Result:

A total of 300 diagnosed thalassemic cases who were registered with thalassemia clinic at a tertiary care institute of North East India during the period of August 2015 to July 2016 were studied.

Types of thalassemia: During the study period, a total of 300 diagnosed thalassemic cases were reviewed. 188 cases (62.6%) had HbE/beta thalassemia,112 cases (37.4%) had beta thalassemia major.

Gender: Out of total 300 multiply transfused thalassemic cases,162 cases (54%)were male and 138(46%) were female. Male to female ratio in this study was 1.17:1

Age distribution: 204 patients (68%) belonged to age group of 1-10 years, 76 patients (25.3%) were in the 11-20 years of age group and in 21–30 years of age group, there were 20(6.67%) cases. The age distribution of patients ranged from 1 to 28 yrs with a mean age of 9.46 yrs (SD=5.63).

Splenectomy : Out of the total 300 thalassemia cases,56(19%) cases are splenectomized.

Age at first transfusion: The mean age at the initiation of transfusion was 1.76 years (SD = 1.66*)*. Age at first blood transfusion ranged from 3 months to 8 years. In eight (2.67%) patients first transfusion was given before 6 months of age and in 292 (97.3%) patients, first transfusion was given after six months of age.

Number of blood transfusion: Total number of units of blood transfused ranged from 6 units to 327 units (mean: 91.65+63.17 units, median 73.0 units). Among the total of 300 thalassemia patients, 96 (32%) had received more than 100 units of blood transfusions, compared with 204 (68%) patients who had received upto100 units.

Prevalence of Alloimmunization in Relation to Mean Age, Age at First Transfusion and Total Number of Transfusion:

The mean (SD) age of thalassemia patients with alloimmunization and without alloimmunization was 16.18 and 9.08 years respectively and this difference was statistically significant($p<0.05$).The mean(SD) age at first blood transfusion in patients with alloimmunization and without alloimmunization was 2.57 and 1.72 years respectively and this difference was statistically significant ($p=0.04$).The mean total number of transfusions of thalassemia patients with and without alloimmunization was 161.62 and 87.71 respectively and this difference was statistically significant.($p<0.05$).

Prevalence of Alloimmunization And Autoimmunization:

Among the 300 patients 5.3% (n=16) patients were diagnosed to have alloantibodies. Autoantibodies were detected in 1 % (n = 3). Among 300 patients, 3 had (1%)developed autoantibodies as determined by positive autocontrol on gel card (IgG + C3d) as well as positive direct antiglobulin tests.

Alloantibody Specificity:

Sixteen patients out of total 300 patients(5.33%) developed alloantibodies. A total of 19 alloantibodies were identified in patient population. In the alloimmunization group, three patients developed dual antibodies while thirteen patients developed single antibody.The most common alloantibody was Rh-related (78.92%; 15 of 19), comprising anti-c (42.1%; 8 of 19), anti-E(26.3%; 5 of 19),anti-C & D (5.26% each; 1 of 19), followed by anti-Kell (10.5%; 2 of 19) comprising of anti-K,anti-Kidd (comprising of anti-Jka 5.26%; 1 of 19 and anti-Jkb 5.26%:1 of 19).Data of patients developing alloantibodies is as shown in Table 2.

DISCUSSION:

Alloimmunization to red cell antigens is an immune response usually stimulated by the transfusion of blood products and is one of the complications of RBC transfusions. The factors that predispose to alloimmunization are complex and involve 3 main contributing elements: the RBC antigenic difference between the donor and the recipient, the recipient's immune status and the immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system.[14,15,16] In this study,among the 300 multiply transfused thalassemia patients,5.33% (n = 16) patients were diagnosed to have alloantibodies. The previous studies have reported quite variable rate of alloimmunization ranging from 3.1% to 37% in patient with different ethnic origin.(Table 3) A low rate of alloimmunization may be expected when there is homogeneity of RBC antigens between the blood providers and recipients. Homogeneity between the patient and blood donors population may be the reason of low rate of alloimmunization in our study.

In this study,the majority of the alloimmunized patients were between 21 to 30 years of age (20%), followed by the age range of 11 to 20(10.5%), 1-10 years(1.96%) respectively. There was significant association between alloimmunization and age in this study($p<0.05$). Most of the cases of alloimmunization (20.0%) were detected in the age group of 21–30 years as those patients were dependent on blood transfusion for several years.Thus it was assumed that the patients who required blood transfusion for several years with multiple units had more chance to form alloantibody in course of their life.

In this study,alloimmunization rate is more in females (7.25%) than male (3.70%), as the value was more than 0.05($p=0.174$),there were no significant differences observed in rate of alloimmunization between male and female patients population. This has been supported by a review study by Esther P et al.[23] which stated that more exposure to immunizing events through pregnancy might be the reason for high rate of alloimmunization in females and this should not be consider as high risk factor for alloimmunization.

In this study,56 of 300(18.6%) patients underwent splenectomy, only 3 of them had alloantibodies .The prevalence of alloantibodies was more (5.36%) in study subjects who have undergone splenectomy.The comparison of the rate of alloimmunization among splenectomized and non-splenectomized patients (5.36% and 5.33% respectively) was not statistically significant ($P>0.05$). In 2015,Dogra et al [24]and in 2016, Shamsian BS et al[16] showed that splenectomy was not a significant factor in the development of alloimmununization.

In this study, the occurrence of antibody formation was more (21.2%) in patients who were hemotransfused for more than 150 times. There was significant difference between patients developing alloimmunization ($P<0.05$) with respect to the number of transfusions. In 2015,study done by Dogra et al[24] found that the alloimmunization rate was seen higher in those who received >12 transfusions (8.82%) as compared to those who received up to 12 transfusions.

In this study,out of 191 patients who were started transfusions at and less than 1 year of age, five developed antibodies (2.73%), while 11 out of 109 (10.0%) patients whose age at the start of transfusions was more than 1 year,developed alloantibodies, It has been previously shown that alloimmunization risk was significantly lower in haemoglobinopathy patients who started transfusion therapy at a very young age (<3 years)compared with those who started later in life,where an immature immune system and some form of the acquired immune tolerance to allogenic RBC antigens is held responsible for the reduced alloimmunization risk[25,26].

In this study alloantibody against c antigen was the most common alloantibody against a single red cell antigen (42.1%) followed by alloantibody against E (26.3%).In 2015,Datta SS[21] et al found that alloantibody against c antigen was the most common alloantibody (28.57%) followed by alloantibody against E (21.42%).Among the alloantibodies against multiple red cell antigens alloantibody against c and E was the most common (12.5%). The present study showed

similar result as described by Thakral B et al[22] and Datta SS et al[21] regarding of specificity of alloantibody.Various other researchers[17,27,28] have reported a higher percentage of anti-E and anti-c in ethnic Asian populations.

In the present study,3(1%) patients developed autoantibodies. Previously studies reported 1.7% to 11% rate of autoimmunization in thalassemia patients[26,103]. No autoantibody was associated with alloimmunization in this study.

CONCLUSION:

Alloimmunization i.e.development of alloantibody against the foreign RBC is one of the important complications of blood transfusions in multiply transfused thalassaemia patients.Alloimmunization was detected in 5.33% of multitransfused thalassemia patients.Rh and Kell blood group system antibodies accounted for more than 80% of alloantibodies. Higher frequency of alloimmunization was found, with increase in number of transfusions and in those who received transfusions after 1 year of age.Patient age was found to be significantly higher in alloimmunized patients than in non alloimmunized patients.There is a need to formulate a balanced and cost-effective approach for transfusion management of thalassemics to minimize alloimmunization and autoimmunization.This study re-emphasizes the need for RBC antigen typing before first transfusion and issue of antigen matched blood (at least for Rh and Kell antigen). Early institution of transfusion therapy after diagnosis is another means of decreasing alloimmunization.Regular antibody screening for already alloimmunized patients to check for the disappearance of old antibodies or development of new alloantibody. These measures will help in decreasing the incidence of RBC alloimmunization and delayed hemolytic transfusion reactions in these patients.

TABLE 1: Showing frequency of alloantibodies in patient's population in relation to mean age of patients, age at first transfusion and total number of transfusions.

Parameter	Alloantibodies(%) Present(a) 16(5.33)	Absent(b) 284(94.6)	P value(a) versus (b)
Mean age(years)	16.18	9.08	0.0001
Mean age at first transfusion (years)	2.57	1.72	0.046
Mean total transfusions	161.62	87.71	0.0001

FIG 1: Pie diagram showing percentage of alloimmunized patients.

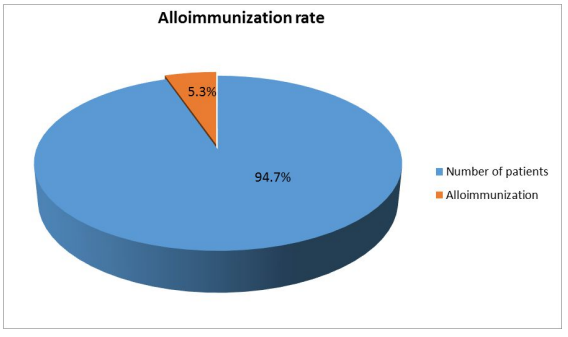

FIG 2: Pie diagram showing distribution of alloantibodies among 16 alloimmunized patients

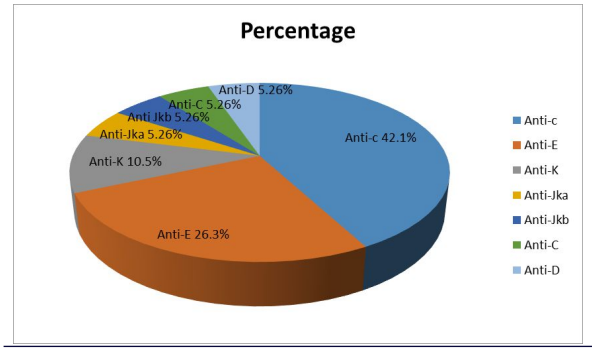

TABLE 2: Characteristics of alloimmunized thalassemia patients

	Age	Gender	Age at the start of transfusion	No.of units transfused	Splenectomy	Antibody specificity
Case 1	6	Male	6 months	64	No	Anti E,Anti-c
Case 2	21	Female	7 years	168	No	Anti-c
Case 3	12	Female	5 years	84	No	Anti-C
Case 4	8	Male	6 months	90	No	Anti-c,Anti-E
Case 5	18	Female	6 months	210	Yes	Anti-c,Anti-K
Case 6	18	Female	2 years	192	No	Anti-K
Case 7	19	Female	1 year	216	Yes	Anti-c
Case 8	13	Male	6 months	150	No	Anti-Jka
Case 9	15	Female	5 years	160	No	Anti-D
Case 10	28	Female	5 years	276	Yes	Anti-c
Case 11	10	Female	3 years	84	No	Anti-Jkb
Case 12	28	Female	5 years	280	No	Anti-E
Case 13	18	Male	3 years	180	No	Anti-c
Case 14	7	Male	6 years	16	No	Anti-E
Case 15	22	Female	2 years	260	No	Anti-c
Case 16	16	Male	3 years	156	No	Anti-E

TABLE 3: Comparative analysis of alloimmunization rate of various studies

Study	Year/Place	Rate of alloimmunization
Spanos et al[13]	1990/Greece	22.6
Sirchia et al[14]	1985/Italy	5.2%
Karimi et al[17]	2004/Iran	5.3%
Bhatti et al[18]	2004/Pakistan	6.84%
Noor Haslina et al[19]	2006/Malaysia	8.6%
Chao et al[20]	2013/Taiwan	9.4%
Dutta SS et al[21]	2015/Kolkata.India	5.6%
Gupta et al[8]	2011/Delhi,India	9.8%
Shenoy B et al[9]	2013/Banglore,India	9.46%
Thakral B et al[22]	2013/India	3.4%
Present study	2016/assam,India	5.33%

REFERENCES

1. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease. Elsevier Health Sciences; 2014 Aug 27.Vol 1,P 638.
2. Deka R,Reddy AP,Mukherjee BN et al.HbE distribution in ten endogamous population groups of Assam.Hum Hered 1988;38:261-6.
3. Colah R,Mohanty D.Beta thalassemia:Expression,molecular mechanisms and mutations in Indians.Indian J Paediatr 1998;65:815-23.
4. Modell B,Berdoukas V.The clinical approach to thalassemia.New York and London: Grune and Stratton;1984.P.332-97.
5. Kattamis C,Touliatos N,Haidas S,Matsaniotis N (1970)Growth of Children with thalassaemia: effect of different transfusion regimens. Archives of disease in childhood. 45:502-505.
6. Hoffbrand AV,Moss AH,Pettit JE.Essential Haematology 5,Edition Oxford:Blackwell Publishing 2005.
7. D.Mohanty & R.B. Colah & A.C.Gorakshakar ((2013)Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study J Community Genet 4:33–42.
8. Gupta R, Singh B, Rusia U, Goyal S (2010) Red Cell Alloimmunization in Routinely Transfused Patients of Beta Thalassemia Major. IJBTI 1: 1-4.
9. Shenoy B, Voona MM, Shivaram C, Nijaguna S. Red cell alloimmunization in multi transfused patients with beta thalassemia major–a study from south India. International Journal of Medical and Pharmaceutical Sciences. 2013;3(10):31-40.
10. Azarkeivan A, Ansari S, Ahmadi MH, Hajibeigy B, Maghsudlu M, Nasizadeh S, Shaigan M, Toolabi A, Salahmand M. Blood transfusion and alloimmunization in patients with thalassemia: multicenter study. Pediatric hematology and oncology. 2011 Aug 22;28(6):479-85.
11. Shah S, Shah M, Gajjar M, Bhatnagar N, Soni S, Shah M. Frequency Of Alloantibody In Multiple-Transfused Thalassemia Major Patients And Factors Influencing On Alloimmunization. National Journal of Integrated Research in Medicine. 2016 Jan 1;7(1):41-6.
12. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, et al. (2000) Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. Blood 96: 3369-3373.
13. Spanos T, Karageorga M, Ladis V, Peristeri J, Hatziliami A, et al. (1990) Red cell alloantibodies in patients with thalassemia. Vox Sang 58: 50-55.
14. Sirchia G, Zanella A, Parravivini A, et al. Red Cell Alloantibodies in thalassemia major. Transfusion 25; 110-112, 1985.
15. Tormey CA, Fisk J, Stack G, Red Blood Cell Alloantibody frequency, specificity and properties in a population of male military veterans. Transfusion 48; 2069-2076, 2008.
16. Shamsian BS, Arzanian MT, Shamshiri AR, et al. Frequency of red cell alloimmunization in patient with beta major thalessemia in an Iranian referral Hospital, Iran J Pediater 18; 149-153, 2008.
17. Karimi M, Nikrooz P, Kashef S, Jamalian N, Davatolhagh ZR. RBC alloimmunization in blood transfusion-dependent β-thalassemia patients in southern Iran. International journal of laboratory hematology. 2007 Oct 1;29(5):321-6.
18. Bhatti FA, Salamat N, Nadeem A, Shabbir N (2004) Red cell immunization inbeta thalassaemia major. J Coll Physicians Surg Pak 14: 657-660.
19. Noor Haslina MN, Arriffin N, IlluniHayati I. Red cell alloimmunization in multiple

transfused Malay Thalassaemic patients. Southeast Asian J Trop Med Public Health 2006; 37: 1015-20.
20. Chao YH, Wu KH, Lu JJ, Shih MC, Peng CT, Chang CW. Red blood cell alloimmunisation among Chinese patients with β-thalassaemia major in Taiwan. Blood Transfusion. 2013 Jan;11(1):71.
21. Datta SS, Mukherjee S, Talukder B, Bhattacharya P, Mukherjee K. Frequency of Red Cell Alloimmunization and Autoimmunization in Thalassemia Patients: A Report from Eastern India. Advances in hematology. 2015 Sep 6;2015.
22. Thakral B, Saluja K, Sharma RR, Marwaha N. Red cell alloimmunization in a transfused patient population: a study from a tertiary care hospital in north India. Hematology. 2008 Oct 1;13(5):313-8.
23. Esther P. Verduin, Anneke Brand, and Henk Schonewille. Is Female Sex a Risk Factor for Red Blood Cell Alloimmunization After Transfusion? A Systematic Review. Transfusion Medicine Reviews, Vol 26, No 4 (October), 2012: pp 342-353.e5.
24. Dogra A, Sidhu M, Kapoor R, Kumar D. Study of red blood cell alloimmunization in multitransfused thalassemic children of Jammu region. Asian journal of transfusion science. 2015 Jan 1;9(1):78.
25. Charache S. Problems in transfusion therapy. N Engl J Med. 1990 ;322 :1666–8.
26. Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. Transfus Med Rev. 2007;21:58–71.
27. Bilwani F, Kakepoto GN, Adil SN, Usman M, Hassan F, Khurshid M. Frequency of irregular red cell alloantibodies in patients with thalassemia major: a bicenter study. JPMA. The Journal of the Pakistan Medical Association. 2005 Dec;55(12):563-5.
28. 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 Nov;43(11):1604-10.