International Journal of Medical Science and Education

www.ijmse.com

Original Research Article

pISSN- 2348 4438 | eISSN-2349- 3208

STUDY OF PRODUCTION OF ALLO-ANTIBODIES IN MULTIPLE TRANSFUSED THALASSEMIA PATIENTS IN PEDIATRIC AGE GROUP (2-16 YEARS)

Dr. Jitendra Kumar Gupta[1], Pawan Kumar[2] ,Usha acharya[3]

1. Assistant professor, 2. Resident, 3. Professor, Department of Paediatrics Mahatma Gandhi Medical College &Hospital, Jaipur, Rajasthan, India

*Corresponding author – **Pawan kuamr**
Email id –- jitendergupta@live.com

Received:16/08/2018 ***Revised:06/09/2018*** ***Accepted:15/09/2018***

ABSTRACT

Background- Thalassemia is a inherited disorder in which abnormal haemoglobin is form which required regular blood transfusion. Due to regular transfusion there are chances of transfusion of unexpected antibodies other than anti A and anti B. Aim-is to detect and subtype these unexpected antibodies in serum of multitransfused patient in Jaipur . Materials & Methods- A total of 100 patients of proven Thalassemia were included in study at Mahatma Gandhi Medical College & Hospital and JK Lon Hospital, Jaipur. Information on transfusion history was recorded, 5 ml of blood was collected from each subject and plasma was separated. These samples were subjected to direct Coomb Test by CGA (Column Gel Agglutination) technique. Result- In the present study, the Allo-antibodies which were most common among the 7 positive samples out of 100 are Anti D, Anti K, Anti E and Anti MN with an incidence of 28.57%, 28.57%, 28.57% and 14.3% respectively. Blood group O had maximum number of Allo-antibodies (57%). According to number of transfusions, Allo-antibodies of Thalassemia major patients showed statistically significant increase with increase in number of transfusion. Conclusion- From the current studies, it can be concluded that Allo-antibodies to minor group also cause hemolysis and they required more frequent blood transfusion.

Keywords: CGA, alloantibody, DCT, thalassemia, paediatric age, mean frequency

INTRODUCTION

The word Thalassemia is derived from Greek thalassa-sea and emia-blood. Thalassemias are heterogeneous group of disorder of genetic origin in which abnormal production of haemoglobin chain. Clinical severity depend on the degree to which the synthesis of globins chain is impaired, altered synthesis of the other globins chains, and co-inheritance of other abnormal globins alleles1. The type of Thalassemia usually carries the name of the chain that is not produced..

Although blood transfusion is most important modality of treatment but it may be associated with blood transfusion releted complication such as iron overload platelet and red blood cells alloimmunization, infection therefore screening for irregular antibody should be a part of all pretransfusion testing2.

Unexpected antibodies are antibodies other than naturally occurring Anti A or B. Such antibodies are found in 0.3-2.5% of population, depending upon

the group of patients or donor studied and the sensitivity of the test method used3.

Immunization to transfused RBC antigen may be due to transfusion, due to pregnancy or injection of immunogenic material. In some instant the immunizing event is unknown 4.

Antibodies specificity and its ability to react at 37 C are two different modality and very helpful in detecting in vivo significance.

According to FDA following antigens present on red cells are permitted for antibody screening :- D, C, E, c, e, M, N, S, s, P, Le, Le, Jk, Jk, Fy, Fy, K, k. For the detection of these antibodies DCT and ICT is done, cell panel is prepared in coombs positive cases.

With the screening and identification technique, the alloantibodies should be identified and patients should be given corresponding antigen negative donor unit. This study help in reduce the transfusion mediated destruction of RBC, thus help in reduction in number of transfusion. Less no of transfusion reduces the psychological and financial burden on the family and will increase compliance of the patient. Aim of this study is to detect irregular antibodies in sera of 100 thalassemia major patient and To subtype such antibodies with particular reference to ant Rh, anti Duffy, anti Kell, anti Kidd, anti Lewis and anti MN group and To know the relationship between number of transfusion and alloantibodies.

MATERIAL AND METHOD

This is Observational cross sectional study of 100 patient in MGMC hospital and JK LON hospital jaipur over one year. This study include male and female both of 2-16 years of age with multiple transfusion(>2/year) who are giving consent. Patients with single blood transfusion and who are above 18 years are not included in this study.Institutional Ethical committee for research on human subjects approved my study and clearance was given to the study protocol. Written informed consent of parents or guardian and assent (for those>12 years) was obtained for participation in the study prior to evaluation of their child.

Selection of patient : Enlisting patient with transfusion dependent beta thalassemia major attending thalassemia ward for regular blood transfusion in MGMC Jaipur.

This study is on already diagnosed 100 thalassemia patient which are on regular transfusion. Information on transfusion history was recorded 5ml blood was collected from each subject and plasma was separated. These patients were subjected for direct coomb test by CGA (column gel agglutination) technique.

Protocol for DCT-

Preparation of cell suspension- add 50 microliter of packed RBC of test sample (washed once in saline) in 1 ml of Normal saline solution and mix uniformly.

Procedure

Open the wells of the Biovue cassette

↓

Add 10 microliter of RBC suspension

↓

Centrifuge the cassette for 5 min using biovue centrifuge

↓

read the results

↓

(Photograph)

Specimen those are positive for coombs test underwent 3 cell panel screening by CGA. Panel cell have the known antigen consisting of antigen as -Rh, kell, duffy, lewis, P1MNS, LUTH. Results were analysed and prevalence of alloantibody were calculated. Chi square test were used to test the

association between different study variant and allo immunization status.

RESULTS

Of the 100 patients, 56% were males and 44% were females with 62% were received 10-40 number of transfusion followed by 25% were received 40-70 number of transfusion and 13% were received 70-100 number of transfusion. The ABO blood groups of the 100 patients were as follows: 33% of patients had blood group A, 27% had blood group B, 31% had blood group O, 9% had blood group AB, 79% were Rh positive and 21% were Rh negative. With regard to the spleen state that none of the patients had splenectomy. Frequency of blood transfusion is number of transfusion received per year. Out of 100 thalassemia major patient, 53% thalassemia patients receiving more than 12 transfusion every year and 47% receiving less than 12 transfusion every year.

Table-1: Frequency of Allo-antibodies

Type of antibody	N (%)
Kell	2
E	2
D	2
Mn	1

The most common allonantibodies were anti D, anti K and anti E followed by anti Mn. The incidence were 2(28.57%), 2(28.57%), 2(28.57%) and 1(14.3%) respectively of total 100 beta thalassemia major patients.

Out of 100 patients, 7 patients having alloantibodies.In our study out of 100 patients, out of 44 female, 4 having having alloantibody (57.1%) and out of 56 male patients, 3 having alloantibody (42.9%). Out of 7 alloantibody patients, 4 were Rh $^{+}$(57.1%) and 3 were Rh $^{-}$(42.9%). In respect to blood group O having maximum no of alloantibody (57.1%) followed by A (28.6%), B(14.3%). Gender, Rh and bllod groups are showing statistically non significant results.

Table-2: Comparison of alloantibodies of thalassemia patients among study variables

		Absent	Present	X^2	P value
Gender	**FCH**	**40 (43)**	**4 (57.1)**	**0.52**	**0.46 (NS)**
	MCH	**53 (57)**	**3 (42.9)**		
Rh	**+**	**0**	**4 (57.1)**	**0.11**	**0.34**
	-	**0**	**3 (42.9)**		
Blood group	**A**	**31 (33.3)**	**2 (28.6)**	**2.83**	**0.41**
	AB	**9 (9.7)**	**0**		
	B	**26 (28)**	**1 (14.3)**		
	O	**27 (29)**	**4 (57.1)**		

Table-3: Comparison of alloantibodies of thalassemia patients with no of transfusion

	Absent	Present	X^2	P value
<50	**72 (77.4)**	**3 (42.9)**	**4.14**	**0.04 (S)**
>50	**21 (22.6)**	**4 (57.1)**		

Out of 7 alloantibody patients 4(57.1%) patients had done more than 50 transfusion as compared to 42.9% of patients who had done less than 50 transfusion. According to no of transfusion, alloantibodies of thalassemia patients showed statistically significant results with no of transfusion.

Table-4: Comparison of alloantibodies of thalassemia patients with no of transfusion

	Absent	Present	X^2	P value
<12	**52 (98.1)**	**1 (1.9)**	**4.52**	**0.03 (S)**
>12	**41 (87.2)**	**6 (12.8)**		
Total	**93**	**7**		

Mean transfusion frequency/year is 12. Out of 100 thalassemia major patients 53 were received less than 12 unit/year, out of these 53 patient only 1(1.9%) positive for alloantibody. Out of 100 patients, 47 were recieved more than 12 unit/year. Out of these 47 patient only 6(12.8%) came with alloantibody. Alloantibody with number of transfusion showing significant result.

DISCUSSION

Only few study in the world have investigated the frequency and cause of alloimmunization. In present study we calculate the frequency of alloimmunization in Jaipur and observed for best possible solution to reduce the risk of alloimmunization in multitransfused thalassemia major patients. Sample were analyzed for detection of antibodies with particular reference to anti Rh, anti Duffy, anti Kell, anti Kidd, anti Lewis and anti MN groups.

As seen the reported alloimmunisation rates in thalassemics from other parts of India vary from 3.79–9.48 %. In present study, frequency of alloimmunization was 7% which is comparable to other centers in different countries like India (Pimpaldara *et al*), Iran, Pakistan, Malaysia and Italy[3]. In present study, anti Kell, E and D was the most common antibody followed by anti Mn. Agrawal A (2016) *et al* revealed alloantibodies in only 2.9% cases which is less than present study[6] . This may be due to abnormally large sample size and patients of other illness other than thalassemia which may included in study.Prevalence was 1.44% for antibody C and K. In Pakistan and Iran anti K was also the most common antibody Some study showing higher prevalence of anti E in asian region. Elhence P (2014) et al did not find significant differences in E (2%) and c (2%) antigen frequency between thalassemia patients and local donor population[7]. The study have included thalassemia patients and antibody E (2%) and C (2%) both were same in frequency. According to El Sewefy DA (2014) et al was reported only one (0.5%) patient of autoantibodies together with anti-Kell and anti-C. However, many previous studies observed higher results exceeding 25%[8].

Most studies stated that relation between number of blood transfusion and antibodies are unknown in thalassemia[9]. The interval between transfusion are not significant and similar interval was observed in all patients. However the interval are shortened after the development of the antibodies due to decreased survival of foreign RBCs.

In current study using the mean number of transfused blood unit as a cutoff, it was shown that mean frequency of blood transfusion were more than 12 per year in alloantibody patient (P<0.03). We also found that the frequency of blood transfusion may be better judged by the transfusion index wherein the annual blood requirement as volume of transfused blood per kg of body weight was calculated[10].

The development of red cell antibodies (Allo as well as autoantibodies) occurs in a variable number of multiply transfused patients. In such condition transfusion become complicated. Sometime

its become hard to find compatible RBC unit because of presence of clinically significant RBC antibodies, transfusion reaction or platelets refractoriness. Present study is an effort to show blood group alloantibody formation in the patient population.

Our study revealed a statistical significant result between the frequency of blood transfusion and presence of alloantibodies. Similar results found by Hassab AM (2008) et al in Egypt[11]. This is due to the fact that RBCs alloimmunization is a common unwanted transfusion effect that occurs in up to 40% of patients depending on the number of transfusion events.

CONCLUSION

Present study concluded that alloantibody to minor group antigens is more frequent finding among thalassemia major patients which need more frequent blood transfusion. Incidence of detected antibodies were-Anti-Kell>anti>anti-E and mostly against the Kell and RH groups with increased mean frequency of transfusion (>12 units/year) were at higher risk of developing alloantibodies. This problem is due to late onset of 1st blood transfusion and blood is transfused without screening for minor group antigen.

REFERENCES

1. Renzo Galanello, Raffaella Origa. Orphanet journal of rare diseases 5 (1), 11, 2010.
2. Nrages Obeidi, MSc, Ali Reza Mankhian. Laboratory medicine, volume 42, issue 10, 1 october 2011, Pages 618-621.
3. Zaccheaus A. Jeremia, Augustina Mordi. Frequencies of maternal red cell antibodies in port Harcourt, Nigeria. Asian journal of transfusionsci. 2011 jan;5(1):39-41.
4. Mehran karimi, MD, Vahid Emadmarvasti. Major causes of hospital admission in beta thalassemia major patient in iran. Iran J Pediatr. 2011 dec; 21(4):509-513.
5. Pimpaldara RP Patel AC, Patel J, Patel J, Pandya AN, Wadhwani S. A study of irregular antibodies in 200 multi transfused patient. Journal of evolution of medical and dental science 2015;4(73)12659-67. DOI:10.14260/JEMDS/2015/1825.
6. Agrawal A, Mathur A, Dontula Sanjana . Study on red cell alloimmunization in multi transfused patient. Global journal of transfusion medicine. DOI:10.4103/2455-8893. 178005.
7. Elhence P (2014) et al. Indian journal of hematology 2014 sep30(3): 187-190
8. Dahlia A EL Sewefy 2014, Mervat A AL feky. Clinical significance red blood cells antibodiesin multitransfused thalassemic patient in Egypt. DOI:10. 4103/1110-1067.148253.
9. Rabeya Yousuf, Suria Abdul Aziz and Chooin Fun Leong. Incidence of red cell antibodies among the transfusion recipient of university kebangsaan Malaysia medical centre. Indian J hematolblood transfuse 2013 jun ;29(2):65-70.
10. Sbyrakis S[1], Karagiorga-lagana M, Voskaki I. Simple index for initiating transfusion in thalassemia. Br, J Haematol. 1987 dec;67(4):479-484.
11. Hassab AH, Sorour AF, Sayed MI, Salama M, Khalid Aly A. Antibody screening in repeatedly transfused patient. The Egyptian journal of immunology 2008;15(2): 1-14