ASSESSMENT OF RED CELL ALLOIMMUNIZATION AMONG MULTI-TRANSFUSED THALASSEMIA MAJOR PATIENTS

DR.B.VIGNESH

DEPARTMENT OF TRANSFUSION MEDICINE

VINAYAKA MISSIONS KIRUPANANDA VARIYAR MEDICAL COLLEGE AND HOSPITAL SALEM

Abstract

Background:

The single gene condition thalassemia is the most frequent in the world. The inheritance pattern of thalassemia is autosomal recessive. It occurs when one or more globin genes are mutated or deleted, resulting in varied degrees of anaemia that can range from minor to life-threatening. 1. When an immune-competent host is exposed to an incompatible antigen, alloimmunization ensues. A number of factors influence how the immune system responds. Carbohydrate antigens frequently elicit immunological responses that are not thymus-dependent. Antigens that are multivalent induce B cells to produce antibodies without the assistance of helper T cells, resulting in the production of antibodies.

Aim:

To assess the prevalence of alloimmunisation to red blood cell antigens among thalassemia major patients who had undergone multiple blood transfusions.

Methodology:

The following are the instruments and reagents used for the study

Anti-Human Globulin Anti-Screening Cells Anti-Human Globulin Anti-Human Globulin Anti-Human Globul (AHG)

• LISS (Liss)

• BSA of 22 percent

• Incubator for dry air

• A micropipette is a small pipette that is used to

• Tips that can be discarded

Antibody screening is carried out using a traditional tube approach with 22 percent bovine serum albumin and enhancing reagents such as low ionic strength saline (LISS). Antibody screening was performed using a Dia-Med three-cell panel.

Materials and methods:

A) Study setting;

The study was conducted among thalassemia major patients who receiving regular packed cell transfusion in Vinayaka Missions Kirupananda Variyar Medical College and Hospital. Salem

B) Study period;

From September 2016 to August 2017.

C) Study design;

Prospective study.

D) Sample size: 30 patients

E) Inclusion criteria:

1. Registered patients of β-thalassemia major patients.

2. Patients who had are on regular transfusion before the commencement of study (minimum of ten transfusions or more).

3. Patient who are on regular transfusion regimen (interval between 3-7 weeks).

4. Patients who have given written consent to participate in the trial.

F) Exclusion Criteria:

1. Patients with thalassemias other than -thalassemia major, such as thalassemia minor and thalassemia intermedia, autoimmune disorders, and all other forms of thalassemia.

2. Patients who did not receive blood transfusions on a consistent basis.

3. Patients who are adamant about not cooperating

The study was started after getting the clearance from the institutional ethical committee and a written consent was obtained from all the patients who were a part of the research. Standard Hb electrophoresis was used to confirm the diagnosis of thalassemia. Patients' clinical transfusion records were collected and entered into the proforma, with special attention paid to age, age at the time of diagnosis, frequency of transfusion, current clinical status, any increase in transfusion requirements, ethnicity, spleen status, TTI screening, iron chelation allo antibody screen, and so on. Anti-red cell alloantibodies were detected by taking blood samples. The serum was separated using conventional blood bank procedures and stored in labelled tubes at -20 C until the tests were run in batches.

Results:

Only one patient had developed alloantibody among the entire study subjects and so the prevalence of alloimmunisation was 3.3%. 10% of the subjects had developed transfusion transmitted reaction and all of them had developed Hepatitis C infection. Mean Hb in the post transfusion was very much improved than the pre-transfusion period and the difference was found to be statistically significant ($p<.05$).

1.Distribution of the study subjects based on the type of allergic reaction during transfusion

Type of reaction	Frequency	Percentage
FNHTR	17	56.6%
No reactions	13	43.3%
Total	30	100%

2. Distribution of the study subjects based on the prevalence of alloantibody among the study subjects

Alloantibody	Frequency	Percentage
Present	1	3.3%
Absent	29	96.7%
Total	30	100%

DISCUSSION:

In a small percentage of multiply transfused individuals, red cell antibodies (including allo- and auto-antibodies) form. Transfusion therapy may become substantially more difficult in such situations. Because of the presence of clinically significant RBC antibodies, it may be difficult to obtain suitable RBC units, and there may be transfusion reactions or platelet refractoriness as a result of alloimmunization. 4Comparison of prevalence of alloimmunisation between the present study and the previous studies on repeat blood transfusions :

Study	**Number of patients**	**Percentage of alloimmmunisation**
Bibishahinshamsian et al. 2008[5]	121	7.1%
Ansari et al. 2008[6]	80	3.75%
M.N. Noor Hashina et al. 2006[7]	58	8.6%
Khalid Hassan et al. 2004[8]	75	22.7%
G. Sirchia et al. 1985[9]	1435	5.2%
Present study	30	3.3%

The present study of 30 thalassemia patients with multiple transfusions The rate of alloimmunization was found to be 3.3 percent. The actors involved in the development of alloimmunization are complex, with at least three major contributors: the antigenic difference between the blood donor and the recipient, the immune status of the recipient, and the immuno-modulatory effect of allogeneic blood transfusions on the immune system of the recipient.

Chaudharyet al,12 Blumberg et al,10 and Hmidaet al.11 were among the first to report a low rate of alloimmunization (5 to 10%). In research by Spanoset al., a high rate of around 20% was observed.

Monitoring of patients for RBC antibodies after transfusion and repeating this after each transfusion episode, ie. 72 hours after the first transfusion ensures that the transitory antibodies are not missed.[12]

Currently, alloantibody screening cells must be obtained from abroad, which comes at a considerable expense when used on a regular basis. When imported to satisfy the needs for typing and screening a large patient population, these cells have a short shelf life, are destroyed during transportation, and are expensive. Using indigenously created cell panels or screen cells and panels manufactured by some Nationalized Blood Transfusion Centers could be another option for resolving these issues. The benefits of extended red cell phenotyping to reduce alloimmunization have been debated in the literature, but cross matching for the Rh and Kell systems, obtained after extended red cell phenotyping of patients and donors from the time of initial transfusion, has been reported to result in a significant reduction in alloimmunization incidence rate. 13 The identification of the RBC antigenic profile among regular repeat donors for the ease of availability of suitable blood for multiply transfused patients is one of the clear benefits of RBC phenotyping. 14

Conclusion:

Alloimmunization prolongs the cross-matching process and may cause therapeutic delays, as well as raising the risk of a transfusion reaction. Routine RBC antibody screening at predetermined time intervals following transfusion, i.e. repeat antibody screening of the patient if the time gap between two transfusions is more than 72 hours, should be ensured to avoid the effects of alloimmunization. Transfusions will be safer if alloimmunization is reduced.

Keywords :

- AHG-Anti Human Globulin,
- Liss – Low Ionic Strength Solution,
- RBC – Red Blood Cell.
- BSA- Bovine Serum Albumin.

References:

1. M. Sengupta thalassemia among tribal communities of India. The Inter net Journal of Biological Anthropology. 2008. accessed on10.9.2017.

2. Pollack W, Ascari WQ, CrispenJF, O'Connor RR, Ho TY. Studies on Rh prophylaxis II. Rh immune prophylaxis after transfusion wth Rh-positive blood. Transfusion 1971;11: 340-344

3.Urbaniak SJ, Robertson AE. A successful program of immunizing Rh-negative volunteers for anti-D production using frozen/thawed blood. Transfusion 1981;21: 64-69.

4.Brecher E. 12th ed. Chap 18,19. United States: American Association of Blood Banks; 2009. Technical Manual; pp. 389–91. 407.

5.BibiSahin et al. Frequency of Red ced cell Alloimmunisation in patients with β - Thalassemia in an Iranian Referral Hospital. Iran J pediatr 2008; 18. 149 – 153.

6.S. Ansari, P. Voosogh and S.Moshtaghian Assessment of Frequency of Alloimmunisation and Erythrocyte Autoimmunisation in Transfusion Dependent thalassemia patients. Actamedica. Iranica, 2008; 46 : 137 – 140.

7.Noor Haslina MN, Ariffin N, IlluniHayati I, Roseline H. Red cell. Red cell auto antibodies among thalassemia patients in Hospital University sains Malaysia. Singapore Med. J 2007; 48:922-925.

8.Khalid Hassan et al. Red cell alloimmunisation in Repeatedly Transfused Thalassemia Major patients. International Journal of pathology.2004;1:16 – 19.

9.G.Sirchia et al. Red cell alloantibodies in thalassemia major. Results of an Italian co-operative study. Transfusion 1985; 25:110 – 112

10. Blumberg N, Peck K, Ross K, Avila E. Immune response to chronic red blood cell transfusion. Vox Sang. 1983;44:212–7.

11. Hmida S, Mojaat N, Maamar M, Bejaoui M, Mediouni M, Boukef K. Risk factors for alloimmunization by patients with sickle cell disease. TransfusClin Biol. 1994;1:27–34.

12.Shukla JS, Chaudhary RK. Red cell alloimmunization in multi-transfused chronic renal failure patients undergoing hemodialysis. Indian J PatholMicrobiol. 1999;42:299–302.

13. Choudhary R, Sukla JS, Pradhan D, Pahi J. Red Cell Alloimmunization in Multiply Transfused Patients. Indian J Hematol Blood Transfusion. 1997;15(Suppl 4):10–2.

14. Cheng G, Hui CH, Lam CK, Hal SY, Wong L, Mak KH, et al. Haemolytic transfusion reactions due to Mi-antibodies;need to include Miltenberger III positive cells in pre-transfusion antibodies screening in Hong Kong. Clin Lab Haematol. 1995;17:183–4

Table 1: DEMOGRAPHIC PROFILE- DISTRIBUTION ACCORDING TO AGE (n=50)

AGE (yrs)	FREQUENCY	PERCENTAGE
<10	3	6%
11-20	3	6%
21-30	7	14%
31-40	6	12%
41-50	7	14%
51-60	9	18%
61-70	10	20%
>70	5	10%
TOTAL	50	100%

ETIOLOGY	TOTAL	MALE	FEMALE
Masseteric hemangioma	1	0	1
Lymph nodes	12	8	4
Nasopharyngeal angiofibroma	1	1	0
Nasopharyngeal carcinoma	1	1	0
Trigeminal schwannoma	1	0	1
Maxillary carcinoma	1	1	0
Mandibular AVM	1	0	1
Buccal carcinoma	4	1	3
Hemangioma buccal space	1	0	1
Vagal schwannoma	2	1	1
Paraganglioma	1	0	1
Lymphangioma	2	1	1
Branchial cleft cyst	2	0	2
Retropharyngeal abscess	1	0	1
Submandibular neoplasm	1	1	0
Submandibular abscess	1	1	0
Tonsillar carcinoma	1	1	0
Base of tongue carcinoma	1	1	0
Laryngeal carcinoma	5	5	0
Adenoids	1	1	0
Visceral space abscess	2	2	0
Prevertebral abscess	2	1	1
Parathyroid adenoma	1	0	1
Adenoid cystic carcinoma	1	1	0
Intraparotid lymph node	1	1	0
Pleomorphic adenoma	1	1	0
Post radiation necrosis	1	0	1
TOTAL	50	30	20

Table 2:

Distribution of neck lesions according to gender

Table 3: Features of neck lesions in ct

MALIGNANT LESIONS	MALIGNANT LESIONS									
	Enhancement		Necrosis		Bone invasion		Vessel invasion		Adjacent space	
	Homogenous	Heterogenous	Negative	Positive	Absent	Present	Absent	Present	Absent	Present
Laryngeal carcinoma	1	4	1	4	3	2	5	0	2	3
Buccal carcinoma	1	3	2	2	3	1	4	0	4	0
Nasopharyngeal ca	0	1	0	1	0	1	1	0	0	1
Submandibular neoplasm	0	1	1	0	1	0	1	0	1	0
Oropharyngeal carcinoma	0	2	0	2	1	0	0	2	0	2
Maxillary carcinoma	0	1	0	1	1	1	1	0	0	1
Lymphoma	3	0	3	0	3	0	2	0	3	0
Paraganglioma	1	0	1	0	1	0	1	0	1	0
Metastatic carcinoma	0	8	0	8	8	0	8	0	8	0
Papillary carcinoma	0	1	0	1	1	0	0	0	1	0
Adenoid cystic carcinoma	0	1	0	1	0	1	1	0	0	1
Subtotal	6	22	7	21	22	6	26	2	20	8
Total	28		28		28		28		28	

BENIGN LESIONS	BENIGN LESIONS									
	Enhancement		Necrosis		Bone invasion		Vessel invasion		Adjacent space	
	Homogenous	Heterogenous	Negative	Positive	Absent	Present	Absent	Present	Absent	Present
Hemangiomas	2	0	2	0	2	0	2	0	2	3
Nasophryngeal angiofibroma	0	1	1	0	0	1	1	0	1	0
Abscess	0	6	6	0	6	0	6	0	2	3
Lymph nodes	2	0	2	0	2	0	2	0	2	0
Lymphangioma	0	2	0	2	2	0	2	0	2	0
Branchial cleft cyst	2	0	2	0	2	0	2	0	2	0
Adenoids	1	0	1	0	1	0	1	0	1	0
Parathyroid adenoma	0	1	0	1	1	0	1	0	1	0
Vagal schwannoma	0	2	2	0	2	0	2	0	2	0
Post radiation necrosis	1	0	1	0	1	0	1	0	1	0
Mandibular AVM	0	1	1	0	1	0	1	0	1	0
Trigeminal schwannoma	0	1	0	1	0	1	1	0	0	1
Subtotal	8	14	18	4	20	2	22	0	16	6
Total	22		22		22		22		22	

Table 4: DISTRIBUTION OF NECK LESIONS IN NECK SPACES (n=50)

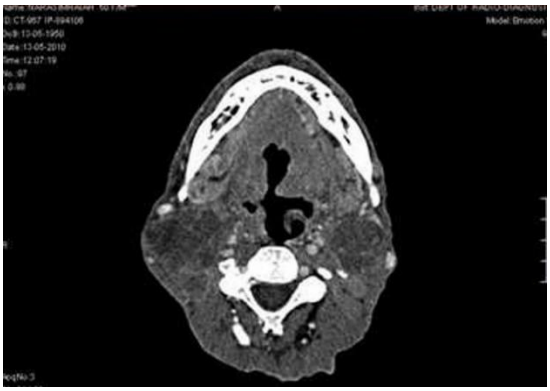
NECK SPACE	NUMBER	%
Masticator space	5	10%
Buccal space	5	10%
Parotid space	3	6%
Parapharyngeal space	12	24%
Retropharyngeal space	1	2%
Prevertebral space	2	4%
Carotid space	3	6%
Submandibular space	3	6%
Visceral space	9	18%
Pharyngeal mucosal space	5	10%
Posterior cervical space	2	4%

Table 5: Sensitivity and specificity of CECT in diagnosing lesions of neck

Lesions according to space	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Submandibular space	100	100	100	100	100	<0.001
Masseteric space	100	97.8	80	100	98	<0.001
Buccal space	80	100	100	98	98	<0.001
Parapharyngeal space	100	100	100	100	100	<0.001
Carotid space	100	100	100	100	100	<0.001
Parotid space	100	100	100	100	100	<0.001
Pharyngeal mucosal space	100	100	100	100	100	<0.001
Retropharyngeal space	100	100	100	100	100	<0.001
Prevertebral space	100	100	100	100	100	<0.001
Posterior cervical space	100	100	100	100	100	<0.001

Visceral space	100	100	100	100	100	<0.001

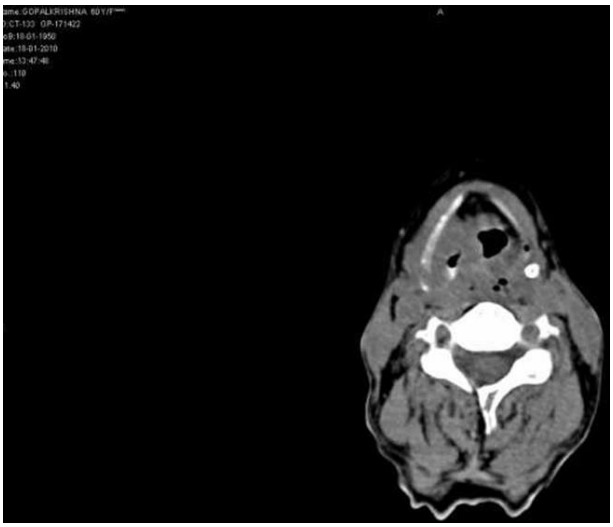
FIG 1.



A 67 year old male with metastatic lymph nodes at level II:

Axial sections of CECT showing multiple hypodense lesions with heterogeneous enhancement with non-enhancing hypodense central areas suggesting necrosis in parapharyngeal spaces bilaterally.

FIG 2.



A 58 year old male with laryngeal carcinoma:

Axial section of CT at thyroid cartilage level demonstrating a lesion with irregular margins and heterogeneous enhancement in the glottis.

Fig 3. Calculation of volume of lesion by MDCT

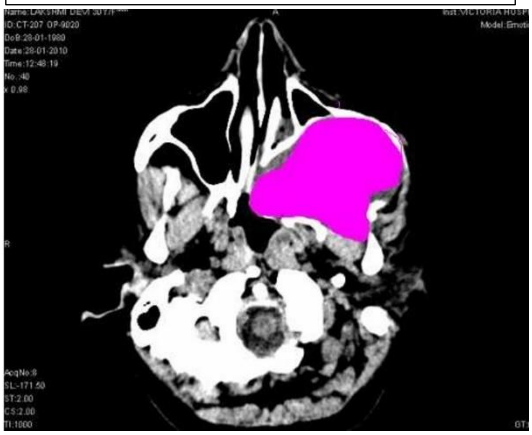


Fig 4. Axial section of CECT demonstrating thickened prevertebral soft tissue with few air pockets suggestive of prevertebral abscess