Original article:
Importance of screening and identification of alloantibodies in multi-transfused patients of thalassemia major.

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Abstract:
Introduction: Thalassemia is a form of inherited autosomal recessive blood disorders characterized by abnormal formation of hemoglobin. These patients need blood transfusion on regular basis to maintain the hemoglobin level in the body. The frequent transfusions received by thalassemia major patients, expose them to the risk of contracting infectious diseases, and development of complication such as iron overload and alloimmunization. The production of antibodies against such alloimmunization induces further hemolysis.

Subject and methodology: The main objective of the study was to find out clinically significant antibodies in multi-transfused thalassemia major patients to prevent hemolysis and to reduce frequency of blood transfusion there by reducing morbidity and mortality. A prospective and observational study comprising of total 205 thalassemic patients were included in the study (females 99 and males 106) in the age ranging from 3 to 43 years who had received more than 10 units of blood within one year. Majority of them were β thalassemia major followed by Eβ and sickle cell disease. Apart from ABO and Rh grouping and issuing of blood by proper crossmatching the alloantibodies were detected by using 3 cell and 11 cell panel by gel technique. Alloantibodies against Rh phenotypes were more than 90%. Discussion and conclusion: Finding of unexpected antibodies must be a part of all pretransfusion testing procedure which will help to accomplish more effective and uneventful blood transfusion of multi-transfused thalassemia patient. Production of alloantibodies in multi-transfused thalassemia patients can be prevented by screening for minor blood groups from beginning in addition to ABO and Rh grouping.

Keywords: Thalassemia, alloimmunization, multi-transfusion.

Introduction:
Thalassemia is a congenital hemolytic disorder caused by partial or complete absence of alpha or beta globin chains and a common genetic disorder distributed throughout the world but more prevalent in south east Asia and Mediterranean countries and India1. Approximately 400,000 babies are born with serious hemoglobinopathies worldwide with about 270 million carriers2. It has been seen that approximately 3% of world population carry beta thalassemia gene. In Europe it was most prevalent in Italy and Greece. Comparatively frequencies between 2.5% and 14.9% have been reported in India3. It was observed that 1000/1.5 million per year live births have β-thalassemia3. In India, nearly 8000-10000 new thalassemia (homozygous) were born every year and more common in Punjabis, Sindhis, Bengalis, and Gujaratis4. The standard protocol for management of β-thalassemia major is regular blood transfusion every 3 to 4 weeks and needs blood transfusion throughout life. In country like India blood transfusion remains only affordable mean for most of the patients. It is the common practice in most of blood banks to issue the blood units in the Eastern part of India by preforming major cross-matching between the recipient’s serum and donor red cells by tube method and by tube AHG method. Usually in most blood banks blood is issued ABO matched compatible blood by tube methods (saline technique). On repeated blood transfusion red cell

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alloimmunization occurs because of antigenic differences between donor and recipient RBCs of the minor blood groups which are not usually tested during cross matching. With the development of alloantibodies following repeated transfusion process leads complications of the patents which needs more transfusion due to hemolytic reaction and significantly complicate transfusion therapy.

Aims and Objectives:
The study was conducted with the aims
1. To find out the antibodies developed due to dissimilarities of Donors and recipients’ minor groups.
2. Hemolysis of such patients can be prevented by properly matched blood transfusion.
3. Repeated transfusion in short periods can be prevented.

Materials and Methods:
The study was conducted from June 2016 to October 2018 in the department of Pathology and Blood Bank, B.S. Medical College, Bankura West Bengal, India after taking permission from Institutional ethical committee. Prospective and observational study was performed. Total 205 transfusion dependent patients between age group of 3 to 43 years (106 males & 99 females) who received more than 10 units of blood both whole blood and PRBC were included in this study. Transfusion dependent thalassemia patient having HIV, HCV and HBV positivity were excluded from the study. Clinical diagnosis, numbers of blood units received with interval of transfusion and history of splenectomy noted.

Transfusion protocol of hemoglobin level maintained around 9gm/dl. All patients were instructed to estimate hemoglobin on regular interval. As per protocol in our institute we start blood transfusion at 7.0 gm/dl and only PRBC were supplied to the patients.

Major and minor coombs crossmatch done by gel technique. The results were noted accordingly. The antibodies (alloantibodies) of all participants were detected by using commercial three-cell panel (Tulip coomb’s gel card & Tulip 3 panel screen cells). All alloantibody screening positive samples were evaluated to identify the specific antibodies. Antibody specificity detection was performed by using Commercial 11-cell identification panel (Jhson and Jhson 11 cell identification panel to detect unexpected antibodies). All the tests were performed using the gel card method by Diamed ID (Switzerland), as per manufacturer’s guidelines and strength of reaction were noted as follows:

Strength of reaction:
• 4+ ---Agglutinated red blood cells form a line at the top of gel micro tube.
• 3+ ----Most agglutinated red blood cells remain at upper half of the gel micro tube.
• 2+ ----Agglutinated red blood cells are observed throughout the gel micro tube. A small button of cells may also be observed at the bottom of the tube.
• 1+ ----Most agglutinated red blood cells remain at lower half of the gel micro tube. A small button of cells may also be observed at the bottom of the tube.
• +/- ----Most agglutinated red blood cells remain at lower third part of the gel micro tube.
• Negative ----All red blood cells form a compact button at the bottom of gel micro tube.
• Mixed field agglutination ---- Agglutinated red blood cells form a line at the top of the gel micro tube. non agglutinated cells for a compact button at the bottom the gel micro tube.
• H ----Haemolyses of red blood cells.

More than 99% of clinically significant antibodies were detected by using three vials set of screening cells. If there was agglutination in the 3 set panel, a set panel of 11 screening cells was required to identify the specific antigen. All results were tabulated, and statistical analysis was performed through SPSS software (version 17.0).

Results:
Among 205 transfusions dependent thalassemia patients were included in the study for detection alloantibodies of which 99 were females and 106 were males. The clinical data of the patients are given below (Table 1 and Table 2).
Table 1. Pattern of different diseases among 205 participants.

| Disease          | No of patients |
|------------------|----------------|
| B thalassemia Major | 139 (67.88%)  |
| E-β thalassemia  | 55 (26.82%)   |
| S-β thalassemia  | 11 (5.3%)     |

Table 2. Distribution of ABO & Rh grouping of the participants.

| Average age of patients | 8.34±7.36years |
|-------------------------|----------------|
| Rh factor | Rh positive – 197(96.1%)  Rh negative -8(3.9%) |
| Blood group A=42(20.5%) | Rh +ve =40 Rh -ve =02 |
| Blood group B=76(38.9%) | Rh +ve =75 Rh -ve =3 |
| Blood group O=68(33.2%) | Rh +ve =66 Rh -ve =2 |
| Blood group AB=17(8.3%) | Rh +ve =16 Rh -ve =1 |

Status of splenectomy

- Splenectomy done =63 patients
- Splenectomy not done = 142 patients

For detection of alloantibodies in the first part of the study screening were done in all 205 transfusion dependent patients of thalassemia major by three cell panels (cell panel I, II, III). Agglutination was seen in 46(22.44%) patients of which alloantibody 27 were females and 19 were males (Female: Male = 1.42:1). The rest 159 (77.56%) patients showed no agglutination in three cell panel indicating absence of alloantibody.

It was noticed that 24 patients show single cell panel positive. Patients showing agglutinations with Cell panel-I, Cell panel -II, Cell panel -III are 9, 5 and 10 respectively. The rest 22 multi transfused patients showed agglutination more than one cell panel either I or II or III cell panel. Group A showed agglutination cell panel I=II= 9. Group B showed agglutination cell panel I +II= 12. Group C showed agglutination cell panel II+III=1.

159 transfusion dependent patient who did not show any alloantibody excluded from the study with advice to complete subgrouping of C, c, E, e and k in addition to ABO and RhD grouping.

Further study was done with II cell panel of 46 patients who had developed alloantibodies and were detected specific antibodies. After testing the 46 patients with II cell panel it was observed that alloantibodies against c was highest (34.78%) and lowest was against k antigen (6.52%). 93.48% of patient showed antibodies against Rh phenotype (Table 3).

Table 3. Incidence of development of different antibodies in study populations.

| Antibody against | Female | Male | Total |
|------------------|--------|------|-------|
| C                | 3      | 3    | 6     |
| c                | 7      | 9    | 16(34.78%) |
| E+C              | 7      | 2    | 9(19.57%) |
| k                | 3      | 0    | 3(6.52%)  |
| e                | 7      | 5    | 12(26.09%) |

142 patients without splenectomy of which 33 patients developed alloantibody whereas 13 patients with splenectomy developed alloantibody. The development alloantibody depends on age. Alloimmunization maximum seen in between age group 10+ to ≤ 15 yrs. (55%) (Table 4).

Table 4. Development of alloantibody age wise.

| Age groups          | Number of Patient(s) | Percentage Among alloimmunized(n=46) | Percentage Among total multi-transfused thalassemic patient |
|---------------------|----------------------|--------------------------------------|----------------------------------------------------------|
| 1-<5years (Group-I) | 2                    | 4.34                                 | 0.97                                                     |
| 5-<10years (Group-II) | 9                   | 19.57                                | 4.39                                                     |
| 10-<15years (Group-III) | 23                  | 50                                   | 24.39                                                    |
| 15-<20 years(Group-IV) | 3                    | 6.52                                 | 1.46                                                     |
| >20years (Group-V)  | 9                    | 19.57                                | 4.39                                                     |

Development of alloantibodies were more in female than male and about 100% alloantibody were against Rh and K blood grouping.

Discussion and conclusion:
Thalassemia is a congenital hemolytic anemia of which thalassemia major patients need lifelong blood transfusion from early childhood.
to raised hemoglobin level and reduces the skeletal deformities associated with excessive erythropoiesis. Although blood transfusion is life saver for thalassemia it may introduce infection which can be prevented by proper transfusion transmitted infection (TTI) testing of blood. Multiple transfusion introduces a multitude of alloantigen of which red blood cell related antigens are most important red blood cellspecific antigens which stimulate the immune system and produce alloantibodies. Although due to improved techniques TTI can control but red cell alloimmunization is still an important factor for reducing hemoglobin spite of extensive research and improved techniques of blood transfusion the red blood cell alloimmunization remain a major problem in transfusion dependent thalassemic patients specially patients from eastern part of India, as pretransfusion antibody screening is not routinely performed.

In the present study we try to evaluate screening and identification of alloantibodies in multi-transfused patients of thalassemia (including Hemoglobinopathy), in Thalassemia unit of B.S. Medical college & Hospital, Bankura. There were several studies in India and different parts of world, but most studies were done in urban areas without any documentation of such studies in rural areas. In a report of Subhra Dutta et al. reported red blood cell (RBC) alloimmunization in 5.6% patients and maximum alloimmunization occurred in the age group of 21–40 years. Azita Azarkeivan et al. in a study (2014) observed 11.3% alloimmunization of which 74% patients were with single alloantibody and 36% patients developed more than one antibody and the most common alloantibodies were anti-Rh antibodies. The present study observed 22.43% alloimmunization out of which 80.43% patient showed single antibody and 19.57% patients developed more than one antibody. About 93.48% of patient showed antibodies against Rh blood group and occurred mostly in the age group of 10-15 years. Reisner et al observed alloimmunization are more with female patients than male.

Incidence and type of alloantibody observed by several workers were different and Rh related alloantibodies were most common alloantibodies. The incident of development of alloantibodies by different workers are shown in Table 5.

Table 5. Incidence and type of alloantibody observed by several workers.

| Study                  | Incidence and Type of Alloantibody |
|------------------------|------------------------------------|
| Bhatti FA et al.14     | Alloantibodies are mainly Rh related |
| HariKrishanDhawan et al15 | 52.17% belonged to Rh blood group system (Anti-E = 17%, Anti D = 13%, Anti-C = 13%, Anti-Cw = 9%), 35% belonged to Kell blood group system, 9% of Kidd and 4% of Xg blood group system. |
| SuvroSantha Datta et al11 | 78.5% of alloantibodies detected were against the antigens of Rh |
| B. Thakral et al16     | Anti-c being the most common specificity (38.8%), followed by anti-E (22.2%), anti-M (11.1%), anti-Le(a) (11.1%), anti-D (5.6%), anti-Jk(a) (5.6%) and anti-Le(b) (5.6%) |
| Eiman Hussein et al17  | The most common alloantibody was Rh-related (37.4%; 46 of 123), comprising anti-E (14.6%; 18 of 123), anti-D (8.9%; 11 of 123), anti-C (8.9%; 11 of 123), and anti-c (4.9%; 6 of 123), followed by anti-Kell (26%; 32 of 123), anti-MNS (9.8%; 12 of 123), anti-Kidd (8.9%; 11 of 123) |
| Present study          | 93.48% are Rh related. The most common alloantibody developed Anti-c (34.78%) followed by Anti-e (20.09%) combined Anti-E +C (19.57%) Anti-C (13.04%), anti-k (5.5%) [all anti-k are female] |

Alloimmunization to red cell antigens is due to immune response usually stimulated by the transfusion of blood products and is one of the complications of RBC transfusions. Developments of anti-red blood cell antibodies still a major problem in thalassemia major patients. In present study showed frequency of red blood cell (RBC) alloimmunization among thalassemia patients with regular transfusions were Rh blood group system antibodies and accounted 93.48% of alloantibodies.

Usually the minor antigens play the role of alloimmunization. Our data shows that the frequency of alloimmunization to minor RBC antigens is high in our multi-transfused thalassemia patients. The high incidence may be due to lack of homogeneity of RBC antigens between the blood
donors and recipients and not matching with minor Rh Antigen.

We concluded that for multi-transfused thalassemia patient identification of alloantibodies must be done for transfusion of well-matched donor (lacking specific antigen against which the antibody developed) for desire rise of hemoglobin level and thereby reduces the transfusion frequencies as well as increase transfusion interval. Blood transfusion is life saving for thalassemia patients and it is the principal method of management of these patients in spite of that it may be associated with complication like RBC alloimmunization. Detection for unexpected antibodies should be a part of all pretransfusion testing procedure which will help to accomplish more effective and uneventful blood transfusion.

**Conflict of Interest:** We declare no conflict of interest among authors.

**Ethical Approval:** We conducted the study after obtaining the ethical approval from Ethical Committee of B.S. Medical College (written permission obtained).

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