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Detection of Alloantibodies in Multitransfused Patients

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Abstract: ***Background: Multitransf used patients may develop alloimmunization to donor 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 these patients. Methods: A prospective cross sectional study was conducted at a tertiary care hospital. Multitransfused subjects who received more than six units of blood (at single or multiple occasions) over a period of three months were enrolled in our study. The study population of 50 cases were broadly divided into three categories Thalassemia, haematolymphoid malignancies and others. 3 cell panel was used for screening and 11 cell panel Column gel agglutination technique was utilised for identification of antibodies. Result: Out of 50 subjects in the study 04 had allo antibodies; 02 cases had alloantibody anti E, one case each had anti e and anti c. One case of Thalassemia had both alloantibody (anti E) as well as autoantibody. Conclusion: Red cell alloantibodies pose a significant problem in multitransfused patients and extended red cell antibody screening is highly desirable in these cases.In our study alloimmunization prevalence of 15.00% was noted in thalassemia patients.***

Keywords: Multitransfused, alloantibodies, columngel agglutination

1. Introduction

1.1 Background

Certain clinical situation like Thalassemia, hematolymphoid malignancy etc require multiple transfusion of RBCs. There are many complications of blood transfusion; Delayed hemolytic transfusion reaction (DHTR) is an important complication of blood transfusion. DHTR is commonly reported in patients with multiple transfusions which at times can lead to hyper haemolysis syndrome and gradually increasing blood demand in chronic transfusion dependent patients. DHTR reaction may be a consequence of secondary immune response due to donor Red Blood Cell (RBC) antigens i.e. allo immunization which is preventable. In a given population, allo immunization depends on the frequency and immunogenicity of blood group antigens and immune status of the patients. (1)

1.2 Red Cell antibodies

Antibodies to RBC are of two types (i) **Alloantibodies**:- which reacts with foreign antigens not present on patient's own RBC, or (ii) **Autoantibodies**:- which reacts with an antigen on the patient's own RBC and with that same antigen on the cells of the other individuals [1, 2, and 3]. Further antibodies to RBC are also classified as (i) naturally occurring: - where antigenic stimulus is unknown. Naturally occurring antibody appears in the serum of the individual who lack corresponding RBC antigen, even in absence of exposure to foreign RBC antigen. ABO blood group antibodies are the typical example of naturally occurring antibodies. These antibodies are generally IgM type and reacts at room temperature. Naturally occurring antibodies are responsible for acute hemolytic transfusion reactions; the incidence of which has declined due to diligent care in sampling at all levels, emphasis on patient identification in transfusion and meticulous cross match test [1, 2, and 3]. (ii) Irregular antibodies or immune antibodies are produced as a result of immunization to foreign RBC antigens either by transfusion of RBC or feto-placental transfusion of blood during pregnancy [3]. These antibodies are IgG type which reacts at 37^0C. Irregular antibodies can transfer through placenta and can lead to Hemolytic Disease of Newborn (HDN). They also pose problems in transfusion during cross match, decreased life of transfused RBC with increase transfusion requirements and at times DHTR. [2&3]

1.3 Present scenario

Presently antibody screening is not practised as a routine procedure in most of the blood banks in India and patients are issued the least incompatible ABO and Rh cross-matched blood for transfusion. With increase awareness for haemovigillance and subsequently as per National AIDS Control Organisation (NACO) guidelines serum of the recipient should be tested for unexpected antibodies with pooled O Rh(D) positive cells or screening red cell panel at room temperature by saline technique and at 37°C by albumin/enzyme as well as indirect antiglobulin test with proper controls (positive, negative and end point). If on screening, antibody/ies are detected, the antibody/ies should be identified by red cell panel, if possible.[4]

2. Aims and Objectives

The study was conducted in a multidisciplinary tertiary care Hospital. A prospective cross-sectional study was conducted in the blood bank of the hospital with the following objectives

a) To detect red cell allo antibodies in multiple transfused patients.
b) To characterise the type of the allo antibodies detected.
c) To determine the relative frequency of occurrence of different allo antibodies in the study population.

3. Materials and Methods

Multiple transfused subjects who received more than six units of blood (at single or multiple occasions) over a period

of three months or more were enrolled in our study. Their pre-transfusion specimens were evaluated for red cell allo-antibodies or auto-antibodies. The study excluded children below 05 yrs, pregnant women and elderly individual more than 60 yrs of age. RBC serology was undertaken using column gel agglutination technique. Commercially available Column gel micro typing system was used for the above study. Basic crossmatch screening was done by using low ionic salt solution (LISS) coomb's card incorporated with AHG (anti human globulin).Each plastic card contained six micro tubes with approximately 35 µl of Sephadex gel prepared in a buffer solution along with preservative. A 0.8 % suspension of donor red cell was prepared by mixing 10 µl of red cell in one ml of LISS (ID diluent).50 µl of the donor's red cell suspension was added to the micro tube, followed by 25 µl of the patient's serum. The card was then incubated at 370 c for 15 mins, and then centrifuged in ID centrifuge at 1175 rpm (85 G) for 10 mins and result read. When there is an antigen antibody reaction the resultant complexes are trapped on the gel surface where as in case of a negative reaction they descend down to the bottom

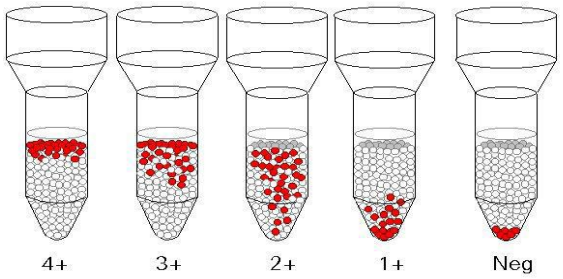

Figure 1: Column gel agglutination, Schematic diagram

While grading the reaction in the gel surface the following scheme was followed. No agglutination indicates that the donor's blood is compatible with recipient and suitable for transfusion. A negative reaction displays pellets of RBCs at the bottom of micro tube with no aggregates in gel matrix. The presence of agglutination indicates incompatibility. Positive reactions are graded from 1+ to 4+. A 4+ reaction is indicated by a solid band of RBCs on top of the gel surface. A 3+ reaction displays agglutinated RBCs in the upper half of the gel column. A 2+ reaction is characterised by RBC agglutinates dispersed throughout the gel column, while a 1+ reaction shows RBC aggregates in mainly lower half of the column. Those giving a positive reaction were screened by commercially available reagent screening cells (3 cell panel) followed by identification cells (11cell panel).

25 µl of test serum and 50 µl of corresponding cell from the cell panel were put in the reaction chamber, incubation done at 37^0c for 15 mins and centrifuged for 10 mins.The reaction pattern in the various gel column were studied under good illumination in naked eye.

A valid consent was obtained from all patient/guardian before enrolling them into the study and due clearance was obtained from the hospital ethical committee. No extra collection of blood was done for any patients. Only the samples received in the blood bank for routine cross matching were utilised for the study.

4. Results and Analysis

The result and analysis revealed following relevant facts

a) Distribution of cases with respect to age and gender

The study group included a total of 50 cases out of which maximum number of cases 22 (44%) were of the age group of <20 yrs, 10(20%) cases were in the age group 20-40 yrs and 18(36%) cases were in the age group of 40-60 yrs. In the present study 32(64 %) of the cases were males as compared to 18(36%) females.

b) Distribution of cases with respect to study groups

The study population of 50 cases were broadly divided into three categories namely Thalassemia, haematolymphoid malignancies and others. The others category included cases like burns, severe anemia, systemic malignancies, end stage renal disease, renal allograft recipient & chronic liver disease. Thalassemia group had 20(40%) cases followed by others category 19 (38%) cases and haematolymphoid malignancy 11 (22%) cases. With a Chi square value 4.38, df=2 and p value=0.11(>0.05) there is no statistically significant distribution in the cases with respect to their diagnosis.

Table 1: Distribution of Cases With Respect to Diagnosis

S No	Diagnosis	Number (%)	Chi square value 4.38
1	Thalassemia	20(40%)	Df=2
2	Hematolymphoid Malignancies	11(22%)	p value=
3	Others	19(38%)	0.11(>o.05)

c) Distribution of cases with respect to blood group

Blood group of the 50 patients was studied by ABO and Rh grouping. Maximum number of study population were of 'B' blood group 22(44%) followed by 'O' group 12(24%), 'A' group 11(22%) and 'AB' group 05(10%).In the Rh system only 01(02%) case was Rh negative while 49(98%) cases were Rh positive. Chi square value for the data is 15.89,df=3 and p value is 0.001(<0.05).Thus there is a statistically significant difference in distribution of cases with respect to blood group.

d) Distribution of cases with respect to number of blood transfusion received

In the study population the cases were grouped according to the number of blood transfusions received into four different clinical groups.

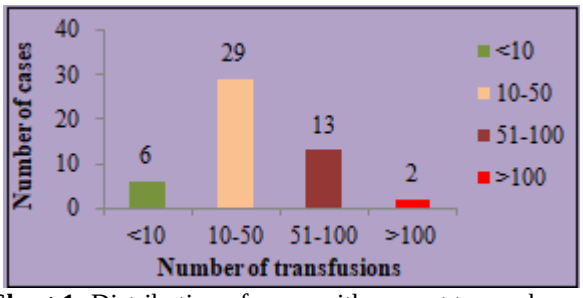

Chart 1: Distribution of cases with respect to number of transfusions

The number of cases in the <10 transfusions group were 6(12%), maximum cases 29(58%) were reported in the 10-50 transfusions group,13(26%)cases received between 51-

100 transfusions and only 2(04%) cases received more than 100 transfusions .Both cases in the above 100 transfusions recipients were thalassemic with highest being 200 units in a girl child. The chi square value for the data is 39.69,df =3 and p value =0.0000 which is highly significant. So, there is a stastistically significant difference in distribution of cases with respect to number of blood transfusions

e) Distribution of cases according to direct antiglobulin test (DAT)

The direct antiglobulin test (DAT) carried out on the pre transfusion blood sample of all the 50 cases revealed that only 04(08%) were positive and 46(92%) were negative for the same.

f) Distribution of alloimmunised cases with respect to other factors

A total of 50 subjects were enrolled for the study; out of 50 cases 04 subjects were detected to have allo antibodies against blood group antigens, out of which 02 individual were detected to have alloantibody anti E ,one individual each had anti e and anti c. One case of thalassemia was detected to have alloantibody (anti E) as well as autoantibody.

Table 2: Alloimmunisation in Respect to Other Factors (Age, Sex, Diagnosis)

Case	Age (yrs)	Sex	Blood group (ABO, Rh)	Diagnosis	No.of tfn	Allo antibody identified
1	09	Male	B Positive	Thalassemia	120	Anti E*
2	46	Male	O Positive	Multiple myeloma	12	Anti c
3	10	Female	B Positive	Thalassemia	200	Anti E
4	09	Female	A Positive	Thalassemia	64	Anti e

g) Alloantibody detection in different clinical groups

Maximum 03(15%) cases of allo immunization were detected in the clinical group of thalassemia followed by 01(9.09%) case in the haematolymphoid malignancy group and 0% in the other group of patients.

h) Allo antibodies detected in various transfusion groups

There was no allo immunized case in the less than 10 transfusions category while there were 01(03.44%), 01(08.33%) and 02(100.00%) allo immunized cases in the 10-50, 51-100 and above 100 transfusions category respectively. Chi square value for linear trends was 9.195 in our study and the p value was 0.00243(<0.05).It can be inferred from the present study that as the number of transfusions are increasing there is a statistically significant increase in antibody positive case detection.

5. Discussion

It is relevant here to carry out discussion about few positive cases of our study

Case-1 (Sample ID 5): This 10 yr old female thalassemic child had difficulty during major and minor cross matching with 6 different ABO matched donor's blood. She had already received about 200 transfusions prior to entering the study. She was in a poor general condition with progressive right heart failure and ascites. An extended Rh& Kell phenotype revealed 4+ reaction with –C,-c,and –e .It showed Mixed field reaction with –E (mostly due to donor RBCs)

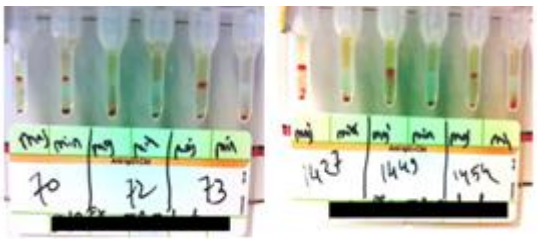

Figure 2: Sample id reaction pattern in major and minor crossmatch, 4+ reaction with six different abo, rh matched donor blood suggesting presence of alloimmunization

Antibody screening shows mixed field reaction in cell panel I and 4+ reactions in cell panel II &III. Antibody identification shows Negative reaction in panel I &4+ reactions in all other cell panels. Auto control was positive. Positive DAT, auto control along with 4+ reactions in most of the cell panel indicates the presence of autoantibody/ies .However allo antibody to recently transfused RBCs may also cause a positive DAT. Due to pan positive pattern in cell panel an alloantibody could not be ruled out. An elution study was carried out and to our surprise we found an alloantibody (anti E).She was offered ABO & Rh matched Rh E negative blood.

Case-2 (Sample ID 14): This 09 yr old male child a known case of thalassemia had received about 120 transfusions; his blood group was 'B'positive. On routine cross matching before issue of blood he had difficulty in cross matching with the donor blood. His Direct antiglobulin test (DAT) was positive (2+). On antibody screening cells (three cell panel) his serum gave a 4+ positivity in panel I& III. The 11 cell panel showed 4+ positivity in panel I, II, III, VII, VIII&IX

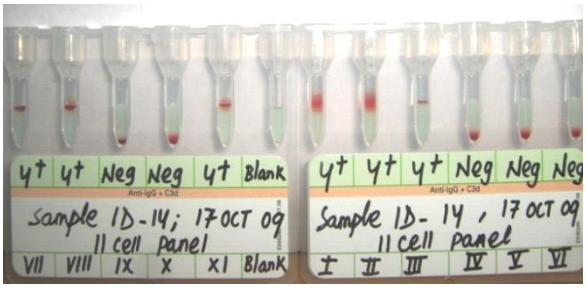

Figure 3: Sample id 14; reaction pattern in 11 cell panel,4+ reaction in cell panel i, ii, iii, vii, viii & xi

By ruling in &ruling out technique the antibody was identified to be **anti-E**. Even though he had received ABO, Rh matched blood he had never been offered an extended Rh matched blood . This probably explains his development of alloantibody E.As per his clinical records his blood demand was also gradually increasing; just to infer that he was having a delayed hemolytic transfusion reaction.

Case No-3 (Sample ID-16): This 46 yr. Old patient was a case of multiple myeloma, who presented with severe anemia. He had difficulty during cross matching with donor blood and his DAT was positive. Antibody screening panel

revealed 4+ positive reaction in cell panel I&III. Antibody identification panel revealed 4+ positive reaction in cell panel II, III, IV, VI, VIII & XI

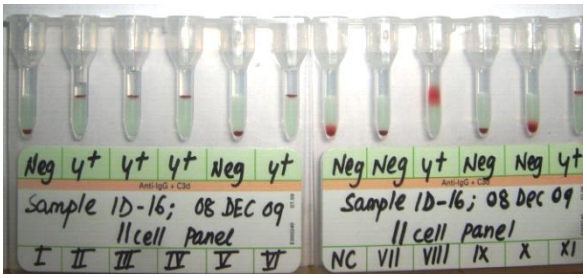

Figure 4: Sample id 16; note the 4+ reaction in cell panel ii, iii, iv, vi, viii & xi

By ruling in and ruling out method the alloantibody identified was anti c .He was subsequently offered ABO and Rh matched Rh c negative blood.

The present study highlights allo immunization being a common problem in multiple transfused patients. **We reported overall allo immunization of 8% in multiple transfusion patients**. **Highest allo immunization of 15% was found in transfusion dependent thalassemia patients** who received regular blood transfusions. **However 9.09% hematolymphoid malignancy subjects were also alloimmunised in our study**. No case was positive in the others category.

Various studies have reported allo immunization in multiple transfused patients. Fluit CR, et al [18] reported allo immunization of 11.8% in multiple blood transfusion recipient's way back in 1990. Overall allo immunization of 8.4% was reported by Redman M et al in transfused patients undergoing elective surgery [29]. Allo-immunization in sickle cell disease has been studied extensively, and various authors reported allo immunization rate of 5-35% [30]. Allo immunization of 37% was reported in thalassemia patients from Taiwan [7]. Similar very high allo immunization of 30% was reported by Ammen R et al from Arab thalassemia patients [17]; and 22% by Singer ST et al in thalassemia patients of Asian descent [12]. Compared to the present study lower allo-immunization was reported in thalassemia patients from Iran- 5.3% [24], Pakistan- 9.2% [31] & 6.8% [20] and Malay- 8.6% [22]. The overall allo immunization of 9% was reported by Schonewille H et al [11] in hematology & oncology cases. Allo immunization of 21.4%, 10.7% and 3.1% in MDS, haematological disorders and end-stage renal failure patients respectively was reported by Stiegler G, at al [32]. Shukla JS and Chaudhary RK from Lucknow reported allo-immunization rate of 9.8% in chronic renal failure (CRF) patients undergoing haemodialysis [33]. The differences in allo immunization were attributed to at least three contributing elements. A) the RBC antigenic difference between the blood donor and the recipient b) the recipient's immune status and c) the immunomodulatory effect of the allogenic blood transfusion on the recipient's immune system. [12,34]. Low rate of allo-immunization is expected in population where there is homogeneity of RBC antigen between the blood donor and recipients [12]; Allo immunization and DHTR can be prevented to greater extent by transfusing extended phenotype matched, leucodepleted red cell units particularly for patient who require long term transfusion support [11,34].

As has been brought out earlier in our study subjects received ABO, Rh D and coomb's cross matched blood. Further they received leukodepleted blood in most of the instances practised at our institution for thalassemics and multitransfused cases. These factors are responsible for relatively low allo immunization in transfused patients. Other factors contributing to the differences in alloinummization are i)the RBC antigenic difference between the blood donor and the recipient ii)the recipient's immune status and iii) the immunomodulatory effect of the allogenic blood transfusion on the recipient's immune system. 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 and leucodepleted blood is being practiced.

6. Conclusion and Recommendation

Overall Allo immunization prevalence of 08% was found in our study population. Allo immunization prevalence of 15.00%, 09.09% and 00.00% was found in Thalassemic, hematolymphoid malignancy and other group patients respectively. Number of blood units transfused was important risk factors for allo immunization. Commonest alloantibody was anti E followed by anti c and anti e found in 50.00%, 25.00% and 25.00% of allo immunised subjects. Extended antigen E,e and c blood group matched and leucodepleted blood transfusion is recommended for patients requiring long term transfusion support. Considering the high rate of allo immunization in Thalassemic, antibody detection should be practiced as a mandatory procedure before every transfusion in all blood banks.

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