ORIGINAL RESEARCH PAPER

Immunohaematology

THE SEROLOGICAL PICTURE OF INADEQUATE RESPONSE TO RED CELLS IN THE MULTITRANSFUSED POPULATION IN EASTERN INDIA.

KEY WORDS: DAT, Immunohematology, multitransfused,

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ABSTRACT

BACKGROUND: Inadequate response to red cell transfusion due to isoimmunisation is common in multitransfused patients.
AIMS: This study aimed to analyse the serological picture and formulate a reasonable approach in these multitransfused patients.
METHODS: This is a prospective study carried over six months on the multitransfused patients referred to the transfusion medicine department of a tertiary care hospital.
RESULTS: There were 147 patients observed, 77 patients were found to be allo/auto-immunised. A majority of them were females[71.43%] and within 20-40 years[46.75%] age group. In 69 of the 77 immunised patients, 38 were positive to both DAT and antibody screen while 31 were only DAT positive; rest 8 patients were only antibody screen positive. A total of 42 antibodies could be identified with 'Anti-E' (38.09%) being the commonest.
CONCLUSION: DAT positivity is a sensitive marker of isoimmunisation. The practice of routine immunohematological workup may prevent certain adverse outcomes.

1. INTRODUCTION:

Repeated red cell transfusion still remains the cornerstone in the management of various diseases like thalassemia, autoimmune hemolytic anemia (AIHA), several malignancies, chronic renal failure (CRF) etc. As per convention, most of these patients receive ABO & RhD compatible units. However these patients are prone to develop various transfusion related adverse events like poor hemoglobin (Hb) increment, acute and delayed transfusion reactions, alloimmunisation and transfusion transmitted diseases (TTDs). Frequency of autoantibodies is also common in these patients. [1]

The present study aimed to analyze the common serological picture which led to inadequate response to red cell transfusion in the multitransfused patients who were referred to us. It was also aimed to identify some of the preventable causes of red cell non responsiveness so as to formulate a reasonable approach .

2. MATERIALS AND METHODS:

A prospective study was carried over a period of 6 months (July'16-Dec'16) on the multi-transfused patients in the department of Transfusion medicine, at a government medical college with the approval of departmental review board.

2.1 Selection of study subjects:

2.1.1: Inclusion of study subjects:

Patients having a history of (a) inadequate post-transfusion hemoglobin increment, (b) increased frequency of red cell transfusion in the last 1 year, (c) development of signs and symptoms suggestive of transfusion reactions or (d) repeated incompatible crossmatches, were included as study subjects.

2.1.2: Exclusion of study subjects:

Patients with history of any ongoing bleeding episode, massive splenomegaly, coagulopathy, or significant drug intake were excluded from the study.

The selected patients were analyzed for medical history (mentioning their onset of disease, transfusion episode, transfusion frequency, date of last transfusion, any adverse events noted during/ after any of the transfusion episodes, cofactors like pregnancy, abortion) and relevant investigation reports in a proper study proforma with their informed consent.

This was followed by the serological workup of their freshly drawn EDTA and clotted blood samples.

2.2. Sample preparation:

Each of the freshly drawn samples was physically verified for any abnormalities like hemolysis /abnormal discoloration, spontaneous agglutination etc. followed by centrifugation at 3000 rpm for 3 minutes to separate red cell from plasma/ serum.

2.3 Laboratory workup:

2.3.1 ABO and RhD grouping:

Forward and reverse grouping was carried out in conventional tube technique (CTT).[2] Any group discrepancy was resolved as per the departmental standard operating procedure (SOP).

2.3.2. Immunohaematological (IH) profile analysis:

Direct antiglobulin test (DAT) and auto control of the patient's sample in LISS was performed by column agglutination technique(CAT) in polyspecific IgG and C3d cards (Matrix Gel System, Tulip Diagnostics Pvt. Ltd., India). Any DAT positive sample was further evaluated for the antibody specificity by using monospecific (IgG / C3d) cards (Matrix Gel System, Tulip Diagnostics Pvt. Ltd., India). Antibody screening of patent's serum/plasma was carried out in monospecific IgG cards by using commercially available 3 cells (Diacell, Bio-Rad, Switzerland). If antibody screen positive, antibody identification was carried out using commercially available 11 cell panel (Diapanel, Bio-Rad, Switzerland). The implicated antibody(s) was identified as per the antigram provided by the manufacturer. [3]

Relevant elution and adsorption methods were done by using commercially available acid elution kit (Bio-Rad, Switzerland) as per manufacturer's instruction in case of DAT positive and antibody screen negative or pan-agglutinating samples. [4]

3.RESULTS:

A total of 147 patients were included in our study of which 74 patients (50.34%) belonged to the age group 0-20 years followed by 46 (31.29%) between 20- 40 years and the rest 27 (18.37%) above 40 years. Eighty eight (59.86%) of them were females rest 59 (40.14%) were males.

Blood group O (56 or 37%) was most frequent followed by B (54 or 36%), A (25 or 16%) and AB (16 or 11%). All the 147 patients were Rh (D) positive individuals and none of them had blood group discrepancy.

3.1. IH profile of the study subjects:

There were 77 patients (52.38%) out of these 147, who were positive either for DAT, auto control, antibody screen or having all of the features suggestive of immunisation. Among them, 55 (71.43%) were females, rest were males.

A total of 36 (46.75%) patients belonged to age group 20-40

years. Figure 1 illustrates a comparative prevalence of immunization in different age groups.

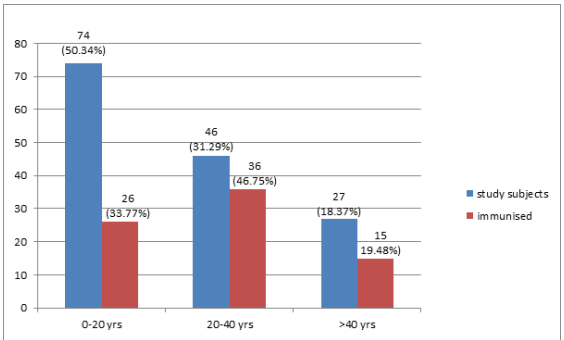

Fig-1 : Comparative age-group distribution of 147 study subjects vs the total immunised patients (n=77) in that age group

In 69 of the 77 positive patients, 38 were positive to both DAT and antibody screen while 31 were only DAT positive; rest 8 patients were only antibody screen positive.

In 38 patients who were both DAT and antibody screen positive, 20 patients showed a strong homogeneous pan reactivity while 18 cases showed a variable pattern in the strength of reaction in antibody screening.

The 20 pan reactive samples did not show any antibody specificity even after adsorption and elution.[figure 2]

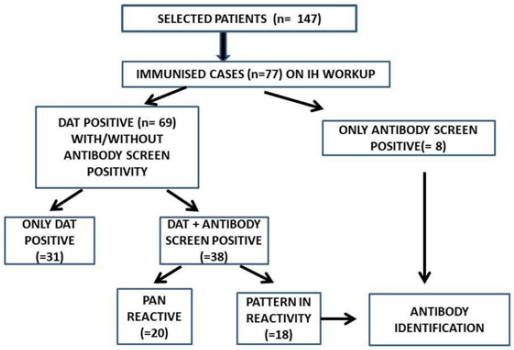

Figure. 2 A brief summary of the IH workup results.

Antibody specificity could be identified in 21 of 26 patients who were either only antibody screen positive (n=8) or positive to both DAT and antibody screen and having a pattern of reactivity (n=18). In the rest 5 of 26 patients' we failed to identify the implicated antibody as the available commercial panel could not specify them.

The summary and profile of these 21 patients with their antibody(s) specificity is given in table 1.

Table 1: Summary and profile of 21 patients in whom antibodies were identified

Sl. No	Age (In Years)	Sex (M/F)	Blood Group	Diagnosis	Antibody
1.	22	F	B+	Thalassemia	c, E, Lu^a
2.	20	M	B+	Thalassemia	Lu^a, K
3.	12	F	A+	Thalassemia	E
4.	19	M	B+	Thalassemia	E
5.	35	F	A+	Thalassemia	E
6.	28	M	A+	Thalassemia	c, E, Jk^b,N
7.	8	M	O+	Thalassemia	E
8.	10	F	O+	Thalassemia	E
9.	10	F	O+	Thalassemia	E
10.	24	F	B+	Thalassemia	c, E
11.	22	F	O+	Thalassemia	E,N, S, Lu^a
12.	23	F	O+	Thalassemia	E, Le^b, Fy^b
13.	20	F	B+	Thalassemia	C, e
14.	20	F	B+	SLE	C, e
15.	24	F	B+	Aplastic anaemia	E, Le^a, Lu^a
16.	10	M	B+	Anemia under investigation	E
17.	24	M	O+	Chronic lymphocytic leukaemia	c, E
18.	26	F	B+	Lymphoma	c
19.	50	F	O+	Chronic renal failure	E, Jk^a
20.	22	F	O+	Anemia under investigation	N, S
21.	85	F	B+	Anemia under investigation	E, K, Jk^b

In 42 antibodies could be identified in these 21 patients, 8 patients having a single antibody, of which anti-E (n=7) is the commonest followed by anti-c (n=1).

The rest 13 patients were having multiple alloantibodies, out of which anti-c+anti-E was the commonest combination (n=4).

4. DISCUSSION:

In our study DAT positivity (46.94%) appeared as the commonest laboratory finding of allo/auto immunization in non-responsive multitransfused patients. DAT positivity was significantly higher in antibody screen positive (38/69) versus antibody screen negative (31/69) individuals ($p<0.00001$ by Chi square test). [5]

We observed that the majority of antibodies were distributed among the thalassemia patients whereas 20 patients who became pan reactive were identified as AIHA after considering their clinical history and presentation as well as their laboratory and peripheral blood smear picture.

Females were having higher rates of immunization (auto/allo); however its relationship with the parity status could not be identified. [6,7].

In our study higher rates of immunization was observed in the age group of 20- 40 years [36/77 (46.75%)] which is consistent with other studies; a lower rate in the pediatric age group may be due to immune tolerance.[8,9]

It appeared immunization to multiple alloantibodies is a more common phenomenon. Anti E appeared to be the commonest offending antibody. Most of the antibodies identified belonged to the Rh & Kell system (64.28%) which are clinically significant and may cause acute/delayed immune mediated transfusion reactions.[10,11,12] We have also identified 3 antibodies belonging to the Kidd system ($1Jk^a$; $2Jk^b$) which has potential to cause anamnestic reactions. Antibodies like anti-N, Lu^a, Le^a are usually clinically insignificant.

The present study has a few limitations, the cause of non-responsiveness to transfusion could not be determined in 70 of 147 patients (47.6%). The other limitations were the unsolvable pan agglutination problems (n=20) and poor resolution of the only DAT positive (n=31) samples. An extensive and multicentric coordinated approach is required.

5. CONCLUSION

DAT appeared to be a sensitive marker of any form of auto/allo-immunisation in patients having inadequate response to red cell transfusion. The spectrum of auto/ allo antibodies showed 64.28% (27/42) of antibodies belonging to the Rh and Kell system with anti-E being the commonest.

The practice of extended Rh & Kell phenotype of the patients before initiation of first transfusion episode and repeated DAT and antibody screen at regular intervals may minimize allo-immunisation in transfusion dependent patients. Also there is a need to develop indigenous cell panels, which can help identify clinically significant allo-antibodies in the Indian population which is not possible with currently available commercial cell panels.

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