

## A PROSPECTIVE STUDY OF DELAYED HEMOLYTIC TRANSFUSION REACTIONS IN A REGIONAL TESTING CENTER – TWO YEARS EXPERIENCE

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### Abstract

**Background:** Delayed hemolytic transfusion reactions (DHTR) are caused by an anamnestic antibody response in the recipient induced by re-exposure to a non-ABO red cell antigen previously introduced by transfusion, transplantation or pregnancy. Antigens implicated most often in DHTRs and DSTRs are in the Kidd, Duffy, Kell, and MNS systems, in order of decreasing frequency. The aim is to evaluate the occurrence of DHTR among transfused patients of the tertiary care centre in South India. **Materials and Methods:** In this prospective study 49450 PRBC and Whole blood transfusions were made during June 2020 to May 2022 received a clinical diagnosis of DHTR or DSTR concurrently with a serologic diagnosis. alloantibody specificity, Hemoglobin, reticulocyte count, serum bilirubin and LDH evaluated as potential predictors of the occurrence of DHTR versus DSTR in different patients. **Result:** Out of 49450 PRBC and Whole blood transfusions 9 DHTR and 1 DSTR cases were found. The following clinically significant antibodies, Anti - D 5, Anti - c: 1 and Anti – E 1, Anti - S, Anti - Fya: 1 and Anti – Jka: 1 were present. **Conclusion:** Our study suggests a significant association between the strength of DAT, the IgG Immunoglobulins either alone or in combination with complements. This study reiterates the importance of acid elution in only DAT positive DHTR cases in identification of the specific alloantibody.

## INTRODUCTION

Delayed hemolytic transfusion reactions (DHTR) are caused by an anamnestic antibody response in the recipient induced by re-exposure to a non-ABO red cell antigen previously introduced by transfusion, transplantation or pregnancy. Mollison defined DHTR as accelerated destruction of transfused red cells that begins only when sufficient antibody has been produced as a result of an immune response induced by the transfusion.<sup>[1]</sup> Since the first case of DHTR attributed to Boorman and colleagues was reported in 1946,<sup>[2]</sup> numerous cases have been described in the literature,<sup>[3-10]</sup> thereafter.

### Most DHTRs share these Characteristics

1. DHTRs generally occur in patients who have been alloimmunized to RBC antigens by previous transfusions, transplants or pregnancies.
2. Because the titer has decreased below detectable levels, the implicated antibody is not detected in

pretransfusion antibody screening or compatibility testing.

3. DHTR is usually suspected 3 to 10 days after transfusion, when clinical symptoms associated with hemolysis are observed and/or serologic findings consistent with DHTR are noted.
4. The clinical symptoms most frequently associated with DHTR include unexplained decrease in hemoglobin and hematocrit, fever, and jaundice.
5. The hallmark serologic findings in DHTR are the development of a positive direct antiglobulin test (DAT) and/or a positive antibody screening test in post transfusion testing because of the presence of RBC antibodies that were not detected in pretransfusion testing.
6. Antibodies directed against Rh (CEce) and Kidd (Jka, Jkb) system antigens are the antibodies most commonly implicated in DHTR; however, numerous other specificities have been described.

The definition of DHTR was clarified by Ness and colleagues in 1990, when the authors introduced the term delayed serologic transfusion reaction (DSTR) to describe cases only with serologic evidence of DHTR (i.e., the development of a positive post transfusion DAT result and a newly identified alloantibody in eluate studies or plasma studies or both), but no clinical evidence of hemolysis.<sup>[11,12]</sup> The authors strictly defined DHTR as a subset of DSTR in which clinical evidence of hemolysis was attributable to a transfusion reaction. Antigens implicated most often in DHTRs and DSTRs are in the Kidd, Duffy, Kell, and MNS systems, in order of decreasing frequency.

Elution removes antibody molecules from the red cell membrane either by disrupting the antigen or changing conditions to favour dissociation of antibody from antigen conducted for the efficacy of various elution methods viz., Acid elution, Glycine-HCl/EDTA, heat elution, Chloroquine diphosphate, and Cold elution, among this elution methods Acid elution method is suitable for eluting both auto and allo Antibodies present on the RBCs.<sup>[13-22]</sup>

In the general transfused patient population, the incidence of DHTRs has been estimated to occur in 1:500 to 1:10000,<sup>[23]</sup> and between 1:10000 to 1:100000 transfusions by some others.<sup>[24]</sup> In combination, DHTRs and DSTRs occur in approximately 1 in 1500 transfusions, with DSTRs occurring up to four times more often than DHTRs. Data from Mayo Clinic calculated an incidence rate of 1:6944 patients transfused for DHTR and 1:3146 patients transfused for DSTR using gel technique and 1:1200 patients transfused for DHTR and 1:611 patients transfused for DSTR using PEG technique.<sup>[22]</sup> Antibodies to E, Fy(a), and Jk(a) were most frequently identified.<sup>[20]</sup> Although DHTR primarily involve extra-vascular hemolysis, some antibodies may fix complement and cause intra-vascular hemolysis. In such instances, hemoglobinuria may also be present, along with elevated serum lactate dehydrogenase and decreased haptoglobin.

DSTR are often first identified in the blood bank, when post-transfusion antibody screen is positive. Close communication with clinical team is indicated to confirm reaction is solely serological and not hemolytic. A complete Immunohematology laboratory is the need of the hour in developing countries; In this context we have initiated a regional testing center for South India at Madras Medical College, Chennai.

#### **Aim**

To evaluate the occurrence of DHTR among transfused patients of the tertiary care centre in South India.

## **MATERIALS AND METHODS**

This prospective study was conducted in the regional testing center for South India at Madras Medical College, Chennai.

We have only included the patient's sample with DAT positivity along with recent history of blood transfusion ( $\leq 15$  days). To investigate suspected DHTR, post-transfusion samples of the suspected DHTR are evaluated for direct antiglobulin (DAT) testing, antibody screening and identification.

If DAT is positive for IgG, then acid elution testing pursued to identify RBC-coating antibody specificity. Additional serologic testing to confirm diagnosis includes repeat antibody screen and identification and DAT on pretransfusion samples (retained in Department of Transfusion Medicine), to ensure previous results were not erroneous. After identifying patient's phenotype on pretransfusion samples and RBCs from transfused product (using stored "segment") phenotyped for antigen corresponding to newly identified antibody. Other tests done in DHTR patients include Hemoglobin, reticulocyte count, unconjugated bilirubin, urine urobilinogen and serum lactate dehydrogenase.

## **RESULTS**

During the study period 49450 PRBC and WB transfusions at regional testing center, A total of 9 cases of DHTR (1:8200) and a single (1) (1:49450) case of DSTR were received.

Out of these 10 cases 6 present within 5 days after transfusion; 3 cases present on 7- 10 days of transfusion. The remaining one case was found out on day 13 on routine cross match. 9 cases of DHTR presented with decrease in HGB among which 2 cases presented with HGB less the 4 gms/dl the remaining 7 cases the HGB was between 7 to 9gms%. The 1 DSTR patient showed only DAT positivity with HGB value of 11gms/dl. Reticulocytopenia was present in 2 patients. The male to female ratio is 4:6. Out of 10 cases 5 (50%) were hematology (2 Thalassemia, 1 Sickle Cell Disease, 1 Aplastic anemia and 1 anemia) patients, 2 Nephrology (1 CKD on dialysis and 1 Post renal Transplant), 3 oncological cases. Previous H/O transfusion is present in all cases (10/10). All the 10 patients were DAT positive, in which mono specific card showed the presence of IgG with C3d among 4 cases and only IgG was found in the remaining 6 cases. 6 out of 10 patients who had only IgG antibodies 5 had hyperbilirubinemia, and 4 out of 10 patients had IgG with complements, they all had elevated LDH along with hyperbilirubinemia. In post transfusion sample of all the 10 patients IAT was found positive in 7 patients. A significant antibody identified in all these IAT positive patients. The antibodies found are Rh blood group system 5 (Anti D 4, Anti c : 1), Anti - S 1, Anti Fya : 1. Acid Elution was done in all 10 cases. Antibody

screening and identification of the eluate showed the presence of following clinically significant antibodies, Anti D 5, Anti c : 1, Anti E 1, Anti Fya : 1, Anti S and Anti Jka: 1.

## DISCUSSION

A total of 9 cases of DHTR (1:8200) and a single (1) (1:49450) case of DSTR were received. General transfused patient population, the incidence of DHTRs has been estimated to occur in 1:500 to 1:10000,<sup>[23]</sup>

6 out of 10 cases present within 5 days after transfusion; 3 /10 cases present on 7- 10 days of transfusion. The remaining one case was found out on day 13 on routine cross match. 9 cases of DHTR presented with decrease in HGB among which 2 cases presented with HGB less the 4 gms/dl the remaining 7 cases the HGB was between 7 to 9gms%. The remaining 1 DSTR patient showed only DAT positivity with IgG antibody with HGB value of 11 gms/dl.; Hyperbilirubinemia was seen in 7/10; LDH rise was seen in 4/10 cases and reticulocytopenia was present in 2out of 10 cases. In the study done by Fasano RM and colleagues In addition to a drop in Hgb, the Hgb A level drops, the lactic dehydrogenase (LDH) rises above baseline, and reticulocytopenia is commonly present.<sup>[14]</sup>In our study DAT was positive in (10/10) 100% patients. All the patients had a history of transfusion. In Fasano miller study the predictive value of a positive DAT was 83% in the patients with IHA, but only 1.4% in the patients without IHA.<sup>[14]</sup> Huub H.vanRossum et al estimated the positive predictive value (PPV) calculated was 10% for DAT and eluate.<sup>[15]</sup>Out of 10 DAT positive patients, mono specific card showed the presence of IgG with C3d among 4(40%) cases and only IgG found in the remaining 6 (60%) cases. Ness and coworkers detected RBC-bound IgG by the DAT in all (100%) patients; however, 56% of them also had RBC-bound complement.<sup>[12]</sup>Similarly O Nathalang et al., in their study found that most of the patients who had IgG antibodies or IgG and C3d coated on their RBC (91.6%) had a history of blood transfusions.<sup>[19]</sup> The antibodies found are Rh blood group system 7 [Anti D 5 (50%), Anti c : 1 (10%) and Anti E: 1(10%)], Anti - S 1(10%), Anti Fya : 1.(10%) anti Jka: 1 (10%)]. Anti Jka and anti Fya were significantly more likely ( $p<0.05$ ) to be associated with DHTR than with DSTR. while Rh system (and other antibodies that do not fix complement in vivo) would be associated with episodes of DSTR. Garratty related the pathogenicity of RBC alloantibodies to specific qualitative and quantitative characteristics of the antibody, some attributes of the target antigen, and the activity of the patient's reticuloendothelial system (RES).<sup>[13]</sup> Of all such characteristics, antibody specificity and thermal range (which are routinely determined at the blood bank laboratory) are the most important. Vamvakas

et al in their study found that the prevalence of DHTR was higher when the implicated RBC alloantibody was anti-Jka with a p value of  $<0.000$ .<sup>[20]</sup> In the study conducted by Ness et al Alloantibody was eluted from the RBCs of all patients.<sup>[12]</sup> Hoeltge and coworkers studied alloimmunization and found the most common specificities were anti- K (23%), anti-E (18%), anti-D (12%), anti-Lea (7.3%), anti-C (6.3%), anti-Fya (5.7%), anti-c (4.4%), and anti-Jka (3%).<sup>[21]</sup>

Acid Elution was done in all 10 cases. Antibody screening and identification of the eluate showed the presence of following clinically significant antibodies, Anti - D 5, Anti - c: 1 and Anti - E 1, Anti - S, Anti - Fya : 1and Anti - Jka: 1. In our study overall rate of informative eluate of DHTR is 100% it may be because we have included only DHTR cases, However the identification of an alloantibody only in eluate is 30%.<sup>[20]</sup> In the study conducted by Ness et al Alloantibody was eluted from the RBCs of all patients.<sup>[12]</sup> In the study done by Mark Yazer Overall rate of informative eluates was 12.7%. 1.7% of these informative eluates were due to DHTR.<sup>[16]</sup> Johnston and Belota described two (2.4%) eluates that revealed a new alloantibody in the eluate which was not in the serum. Both of these patients suffered a delayed hemolytic reaction.<sup>[17]</sup>

## CONCLUSION

Our study suggests a significant association between the degree of DHTR, strength of DAT and the presence of IgG Immunoglobulins either alone or in combination with complements. This study reiterates the importance of acid elution in only DAT positive DHTR cases in identification of the specific alloantibody. For, all hematological especially Thalassemia and Sickle Cell patients who depend on RBC transfusion as a life saving measure, phenotype matched blood from the day of diagnosis will reduce the incidence of alloimmunization. To prevent DHTRs and DSTRs, AABB Standards mandates permanent preservation of all records of potentially clinically significant antibodies and review of previous records before RBCs being issued for transfusion. Once clinically significant antibody has been identified, the patient should receive offending antigen-negative units for all future RBC transfusions. The 3-day interval requirement for RBC type and screening of recently transfused or pregnant patients is based on the finding that anamnestic antibody responses may occur within 3days of transfusion.<sup>[21]</sup>

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