Case Report

Autoimmunization in Thalassemia: A Case Report with Review of Literature

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ABSTRACT

Alloimmunization has been variably reported worldwide in multi transfused thalassemic patients and leads to delay in issue of compatible blood. However, there is paucity of literature on the frequency of red cell auto-immunization in thalassemics, particularly of Indian origin.

We present a case of 4 year old female child, known case of thalassemia major, who presented to the pediatric emergency with impending congestive heart failure. The EDTA sample showed auto-agglutination on naked eye at room temperature which persisted on incubating the sample at 37°C.There was discrepancy in forward and reverse blood grouping at room temperature which was resolved by extended blood grouping at 3 temperatures (4°C, 22°C & 37°C) and patient's blood group was confirmed to be B positive. The polyspecific direct antiglobulin test (DAT) was strongly positive with 4+ reaction and DAT profiling revealed presence of IgG and C3d on red cells. The antibody screening and identification [Biorad ID Diacell I, II & III and Diapanel (11 cell panel)] respectively showed a panagglutinating reaction in Coomb's phase at 37°C. The titers for IgM antibody in saline phase were found to be clinically non significant.

Multiple units put up for the cross match were all incompatible. However, she was given profile matched ("c"neg, "E"neg and "Kell"neg) least incompatible blood slowly, under strict clinical supervision after the written consent from the clinician.

Thus, guidelines for transfusion policy for thalassemic child need to be formulated to minimize the risk of immunization. Rh and Kell matched blood for thalassemic children (better match) has been shown to reduce the rate of immunization in thalassemic children.

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Introduction

Thalassemia is genetically inherited group of disorders that result from reduced rate of production of one or more of globin chains.[1] Survival of thalassemic child is dependent upon regular blood transfusion therapy. While blood transfusion therapy, on one hand improves longevity; on other hand causes complications like red cell immunization. Alloimmunization is a known complication in multi-transfused thalassemic patients and leads to delay in issue of compatible blood. Autoimmunization i.e. formation of antibodies against self antigens is less commonly reported in thalassemics. Hemolysis of patients own red cells as well as transfused red cells occur due to presence of autoantibodies in these patients. As auto-agglutinins are commonly pan-agglutinins in nature, finding compatible blood units for the patients becomes very cumbersome.

Case Report

We present a case of 4 year old Sindhi, female child, resident of Gwalior, who presented in the Pediatric emergency of Kalawati Saran Children's Hospital with complaints of high grade fever with chills and rigor, on and off along with pain abdomen and vomiting since last 1 month. On general physical examination, patient had severe pallor, icterus, tachycardia and tachypnea (heart rate – 150/ min and respiratory rate – 52/min). On per abdomen examination, child had hepatosplenomegaly (5.5cm & 4cm respectively). Past clinical history revealed that child was a known case of thalassemia major, diagnosed at the age of 2½ years [High performance liquid chromatograph (HPLC) of the child was suggestive of beta thalassemia major (HbF 98.2%, HbA 1.7% and $HbA_2$0.5%) while HPLC findings of the mother and father were consistent with beta thalassemia trait with HbA_2 values of 5.3 and 6.1, respectively].

Since then child was on regular blood transfusion at interval of 4-5 weeks from Gwalior and was clinically stable. For the last 1 month child also had history of increased requirement of blood transfusion.

Complete haematological and biochemical investigations were done. The hematological findings revealed severe anaemia with macrocytic normochromic red cell indices and increased reticulocyte count (Hb 2.5 g/dl, reticulocyte count 7%). Biochemical investigations revealed elevated levels of total and indirect bilirubin, 3.8 gm/dl and 2.26 gm/dl, respectively. Thus, both hematological and biochemical investigations were suggestive of hemolytic anaemia. Viral markers for HCV, HBV & HBsAg, Rapid malarial antigen test were nonreactive and urine examination was within normal limits.

EDTA sample was received at Regional Blood Transfusion Center, Lady Hardinge Medical College, for cross match to issue packed red cell. Sample showed auto-agglutination on naked eye which persisted on incubating the sample at 37 °C. Also, there was evidence of hemolysis in the sample at 37° C. Thus, a repeat fresh sample was taken under strict warm conditions and the plasma and the red cells were immediately separated , which also showed auto-agglutination.

There was discrepancy in forward and reverse blood grouping at room temperature following which an extended blood grouping was repeated at 3 temperatures (4°C, 22°C, 37 °C) after multiple washing of red cells. This showed the presence of saline reacting antibodies at 4° C and 22° C. Thus, patient's blood group was confirmed to be B positive (Table 1). The polyspecific direct antiglobulin test (DAT) was done on thoroughly washed red cells and was strongly positive with 4+ reaction (Control Neg) (Figure 1).

DAT profiling revealed presence of IgG and C3d on red cells and IgM showed a mixed field (MF) reaction. (Table 2, Figure 2).The Rh profile of the patient was also done and the results are shown in (Table 3, Figure 3).

The antibody screening and identification [Biorad ID Diacell I, II, III and Diapanel (11 cell panel) respectively] showed a panagglutinating reaction in Coomb's phase at 37 °C. Autocontrol was 4+ positive indicating the presence of autoantibodies (Figure 4). The titres for IgM antibody at 4 °C in saline phase were found to be clinically non significant.

Simultaneously, multiple units put up for the cross match were all incompatible. As the patient was in impending congestive heart failure, she was given profile matched (" c" neg ,"E" neg and "Kell" neg), least incompatible blood. Instructions were given to transfuse blood very slowly, under strict clinical supervision, after the consent of the clinician.

Presently the Hb of the patient is 11.7 g/dL & serum ferritin levels are 1435 µg/L. Patient received intravenous immunoglobulin and is presently on T. Desirox along with prednisolone 10 mg, folic acid & calcium supplements.

Discussion & Conclusion

Blood transfusion is the mainstay of treatment in the thalassemic patients. Autoimmunization in thalassemic child makes transfusion management complicated. Most of the autoantibodies formed are pan-agglutinins in nature and react with all the cells of the panel, as was in our case. Also, in presence of autoantibodies there is increased transfusion rate and need for immunosuppressive

Table 1: Extended Blood Grouping at 3 temperatures (4°C, 22°C, 37 °C)

TEMP.	ANTI-A	ANTI-B	ANTI-D1	ANTI-D2	NS	A CELLS	B CELLS	O CELLS	A/C	CORD BLOOD	BLOOD GROUP
4° C	Weak	4+	3+	3+	1+	4+	2+	1+	2+	Neg	Invalid
22° C	Weak	4+	3+	3+	1+	4+	weak	Neg	Neg	Neg	Invalid
37° C	Neg	4+	3+	3+	Neg	4+	Neg	Neg	Neg	Neg	B+

Table 2: DAT profile of the patient

IgG	IgA	IgM	C3d	C3c	CONTROL
4+	Neg	MF	4+	Trace	Neg

Table 3: Rh profile of the patient

C	c	E	e	k	Control
4+	Dp (4+/neg)	Dp (4+/neg)	4+	Neg	Neg

Fig. 1 : Blood Group (B+) and DCT of the patient (DCT- 4+, CONTROL- Negative)

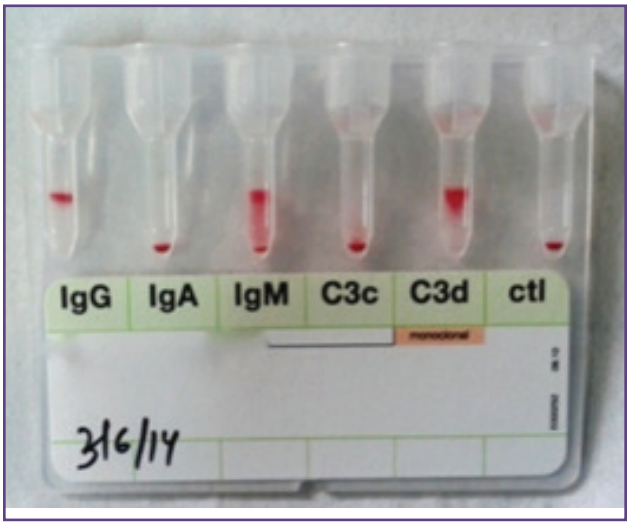

Fig. 2 : DAT profile of the patient

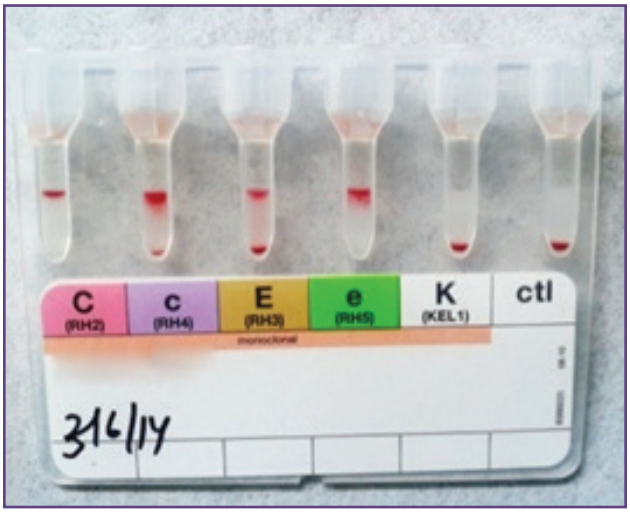

Fig. 3: Rh profile of the patient

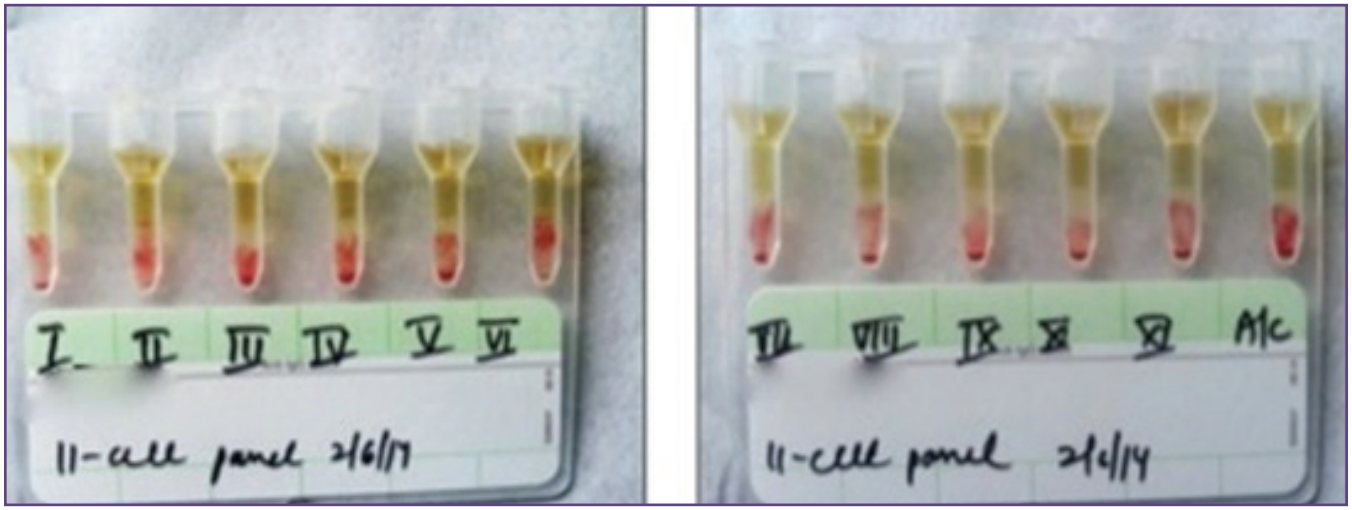

Fig. 4: Antibody identification [Biorad Diapanel (11 cell panel)] - Panagglutinating reaction in Coomb's phase at 37 °C with autocontrol 4+ positive

drugs, splenectomy/ alternate treatment regimes.[2] Alloimmunisation in thalassemia is a well recognised phenomenon and frequency ranges from 3.7% - 30% worldwide. [2-8] However, autoantibodies have been less frequently reported in literature.

Harikrishan Dhawan etal.[3]in 2014, studied frequency of red blood cell alloimmunization and autoimmunization among 319 multiple transfused patients with β thalassemia major and reported very high rate of autoimmunization 28.2 %.

A cross sectional study was also conducted at our Regional Blood Tranfusion Center in 2010 among 211 multitransfused thalassemic children, and the frequency of alloimmunzation and autoimmunization was found to be 3.79 % and 0.47% respectively.[4]

In 2008, Singer etal.[2]reported very high rates of alloimmunization and erythrocyte autoimmunization in 64 transfusion dependent thalassemia patients of predominantly Asian descent i.e22% (14/64) and 25 % (16/64) respectively. Out of these 16 patients with autoantibodies, 3 had severe hemolytic anemia, 11 had IgG while 5 had IgM antibodies These high rates were mainly due to heterogeneity of red cell antigens between donors and recepients (white donors and Asian recepients).

Similar study was done by Noor Haslina etal.[5] in 2006, and studied antibody formation in 58 Malaysian multiple transfused thalassemic patients and found overall frequency of alloantibodies and autoantibodies to be 8.6% and 1.7% respectively; with only a single pan-agglutinating autoantibody.

In 2004, Khalid Hassan et al. [6]conducted a study on 75 cases of multiply transfused thalassemia major patients and found alloimmunization in 22.7% of the patients with absence of autoantibodies.

Ameen etal. [7] in2003, studied 190 transfusion dependent thalassemia patients in Kuwait for red cell alloimmunization and autoimmunization and reported clinically insignificant autoantibodies in 11% (21/109). Among them, 48% had both IgG and C3d while 52.38% patients had only IgG. All these patients with autoantibodies had underlying alloantibodies except in one case. The high incidence of autoantibodies was due to heterogeneity of population and transfusion of ABO and Rh (D) only matched blood.

Michail-Merianou et al. [8]in 1987, compared the frequency of alloimmunization between the usual–match group [ABO and Rho(D) antigens] and the better-match group (ABO, CcDEe & K antigens) which was found to be 23.43% and 14.28% respectively. They did not comment on autoantibodies.

Erythrocyte autoantibodies may cause clinically significant hemolysis by decreasing the red cell survival in vivo. They may lead to difficulty in cross matching blood and delay in transfusion. Das et al.[9] opined that decision to transfuse patients with autoimmune hemolytic anemia should be based on the clinical condition of the patient. No critical patient should be denied blood transfusion due to serological incompatibility. They have suggested that alloimmunization and splenectomy increases the incidence of formation of autoantibodies. Leucoreduction has definitive proven role in reduction of alloimmunzation and autoimmunization. Rh and Kell matching (better match) has been recommended by several authors to reduce the risk of alloimmunization.[8]

We need to frame the guidelines for transfusion policy for thalassemia children. All transfusion services should follow the definite protocols and perform the minimum test required to issue safe and best match leucoreduced packed red blood cells.

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Competing Interests
Nil

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