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Case Report

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Hyperhemolysis Syndrome in a Patient With B-Thalassemia Due to an Anti-Jka Alloantibody

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Abstract

Hyperhemolysis syndrome (HS) is a delayed type of transfusion reactions (DTHRs). These may occur in patients with hemoglobinopathies due to destruction of red blood cells (RBCs). A 17-year-old boy with B-thalassemia and a history of multiple transfusions, after several RBCs transfusions developed hemolysis and hemoglobinuria with a decreased hemoglobin (Hb) level in the absence of bleeding that continued with splenomegaly. The antibody screen test (LISS AHG) and direct anti-globulin test (DAT) were performed. An 11-cell antibody identification panel (IBTO home-made) was used. Acid elution procedure was done using a commercial kit. The patient RBC phenotyping was attempted by treating the cells, following a chloroquine treatment procedure. Laboratory findings showed that his blood group was "A" Rh positive. His RBC phenotyping was negative for Jka antigen. The LISS antibody screening test result was negative with a weakly positive auto control result but DAT was positive for both C3d and IgG. The antibody identification test showed the presence of anti-Jka alloantibody and also anti-Jka was identified with eluate reacting stronger with homozygous cells (Jka+Jka+). The patient received steroid and IVIG as the main treatment and followed by receiving compatible blood units but hemolysis was not resolved and his Hb level was not increased, finally he was splenectomized. The patient's Hb level and clinical symptoms improved after the splenectomy, showing that the spleen may have a role in the destruction of the recipient and the donor blood cells.

Keywords: Thalassemia major; DAT; Anti-Jka; DHTR; Hyperhemolysis

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Introduction

Hyperhemolysis is a delayed type of hemolytic blood transfusion reaction [1] reported in sickle cell anemia [2] applied when pre-transfusion hemoglobin (Hb) level is greater than post-transfusion level. This phenomenon is described in beta-thalassemia also and rarely in patients without hemoglobinopathies [3]. The exact mechanism is not well known but it involves destruction of both transfused donor cells and recipients' red blood cells (RBCs) [4, 5] via alloimmunization. Azarkeivan et al [6] reported that alloimmunization rate in 441 thalassemic patients was 11.3%.

They found that there was a significant association between antibody production and history of transfusion reactions.

Case Report

A 17-years-old male college student with thalassemia major from the North West province of Iran was referred to the Tehran Thalassemia Clinic with compliant of decreased transfusion interval and low Hb level. Since he was 6 months old he was diagnosed as B-thalassemia patient and was on regular blood transfusion every 30 days with the RBCs that were not typed. The transfusion reaction was worse since 1 year ago with decreased Hb level, dark urine and decreased transfusion intervals. In his native city, he was admitted in the hospital, was injected by IVIG and used prednisolone before every transfusion (three units every 15 days) but no improvement was observed. Finally, he was referred to the Tehran Thalassemia Clinic for further diagnosis and treatment. Clinical exams and laboratory tests in the clinic showed that his hematocrit and Hb level were 24.9% and 8.7 g/dL, respectively, in spite of blood transfusion in 2 days prior to the admission. Splenomegaly (5 cm below costal margin) was found in his clinical exam that was confirmed by abdominal sonography that showed a huge spleen (190 mm span) with multiple stones in his gall bladder.

ABO blood typing was done (Anti-A, Anti-B & Anti-D blend IBRF holding Co., Tehran, Iran) using automated method (Hemos II Bio-Rad, Cressier FR Switzerland). The antibody screening test with LISS (low ionic strength saline, DiaLISS; Bio-Rad, Cressier FR Switzerland) as enhancement media and

Table 1. Antibody Screening Test Results With LISS Enhancement

Incubation phases	IS	37 °C LISS	AHG LISS	CCC
Cell I	0	0	0	NT
Cell II	0	0	0	√
Cell III	0	0	0	√
AC	0	0	1+	NT

IS: immediate spin; AHG: anti-human globulin; CCC: Coombs control cells; AC: auto control; NT: not tested. Cell followed by a Latin number indicates an antibody screening cell. √: positive reaction (1^+ - 2^+).

AHG (AHG; CE-Immunodiagnostika, Am Seerain 13 Germany, Eschelbronn) manual technique and acid-eluate test with a commercial kit were performed (Red Cell-Eluate Kit/ Lorne laboratories Ltd., Lower Earley, UK). The eluate was used to identify any suspected alloantibody to red cell antigens attached to the cells but not present in the patient's plasma. Direct antiglobulin test (DAT) was performed using automated technique DC-Lys EM® (Diagast 251/AV.AVINEE-59120 Loos, France). An 11-cell antibody identification panel (IBTO home-made (IBTO-Homemade 11 Cells ID Kit, Registration No. 63882) was used. Chloroquine treatment procedure was used for the patients RBC phenotyping (JUDD's methods in Immunohematology 3rd ed AABB 2008).

Patient's ABO blood group was shown to be an "A" and Rh (D) positive. Panel cells for antibody identification showed specificity for anti-Jka. His direct Coombs test (DAT) was positive for both IgG and C3d.

The antibody screening test was negative with LISS as enhancement media and AHG manual technique, and the results are summarized in Table 1. The auto control showed auto antibody presence.

DAT, using automated Diagast kit, is constituted by a well of antiglobulin anti-IgG, a well of antiglobulin anti-C3d and a well of negative control. The differential DAT was positive as it is shown in Table 2.

Table 2. DAT Result: Automated Technique

IgG	C3d	Negative control
4+	3+	0

Due to patient's history of frequent RBCs transfusion and positive DAT results, an acid elution procedure was performed using a commercial kit. The eluate was tested against the cells of a home-made identification panel that is shown in Table 3.

Anti-Jka was identified with eluate reacting stronger with homozygous cells (Jka+Jka+).

A complement reaction in DAT test results was probably indicative of an anti-Jka complement dependent alloantibody.

Despite the fact that the patient had recently received blood transfusion and the anti-IgG in DAT test was positive, patient's RBCs were phenotyped and found to be Jka negative (Table 4).

One week after the admission his Hb level was 7 g/dL, in the absence of bleeding, in addition to treatment with prednisolone and IVIG and receiving one phenotype-matched RBC unit. His clinical condition did not improve and his Hb level was not rising. Finally the splenectomy procedure was done after consulting with an experienced surgeon. The patient underwent emergency surgery for splenectomy and cholecystectomy; after surgery his condition was better and his Hb level was slowly increased to 8.3 g/dL during 14 days.

Discussion

Chronic multiple transfusion causes alloimmunization frequently [7]. Rate of alloimmunization in thalassemia is 4-37% in comparison to general population (1-4%). RBC antigen difference between recipient and donor is the main cause of alloimmunization [8] that may cause hyperhemolysis syndrome

Table 3. Acid Elution Test Results

Cell No.	Rh-hr	Kell	Duffy	Kidd		Lewis	MNS	P_1	Lutheran	Xg^a	AHG	CCC	Control (last wash)	CCC
				Jka	Jkb									
Cell 1				+	+						W+	NT	0	√
Cell 2				+	0						2+	NT	0	√
Cell 3				0	+						0	√	0	√
Cell 4				0	+						0	√	0	√
Cell 5				+	+						1+	NT	0	√
Cell 6				+	0						2+	NT	0	√
Cell 7				+	0						2+	NT	0	√
Cell 8				0	+						0	√	0	√
Cell 9				0	+						0	√	0	√
Cell 10				+	0						2+	NT	0	√
Cell 11				0	+						0	√	0	√

Cell followed by a Latin number indicates an antibody screening cell. AHG: anti-human globulin; CCC: Coombs control cells. √: positive reaction (1^+ - 2^+).

Table 4. Patient Phenotype Post-Chloroquine Treatment

C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
+	+	mf	+	0	+	+	0	0	+	+	+	+	+

(HS). Alloantibody production occurs in DTHR that destroys transfused RBCs; delayed hyperhemolysis is suggested to be a subset of DTHR with destruction of both donor and host RBCs [3].

HS first was known in patients with sickle cell anemia [4, 5], and it is rarely reported in thalassemia. Several theories are suggested to explain the hemolytic destruction of RBCs like: macrophages hyperactivation, defects in complement regulation, HLA antigen-antibody reactions, a bystander hemolysis and suppression of erythropoiesis [2, 9, 10]. Anti-Jka causes over one-third of DHTRs [8] may be severe. In the absence of HLA antibodies and RBC alloantibodies, it is suggested that macrophages are involved in RBCs destruction [10]. Morawakage et al (2009) reported a case of HS in a child with thalassemia with 2+ DAT for C3b but they could not find any antibody in the patient's serum. They discuss that IgA may lyse RBCs while common serologic tests are negative. They explain that ADCC may occur with low level of antibodies that were not detectable by serologic tests [4].

Laboratory and clinical finding shows that our patient seems to be a case of delayed hemolysis, with post-transfusion Hb level lower than pre-transfusion and a DAT positive. Santos et al [5] described two acute (with negative DAT) and chronic (with positive DAT) forms of HS. Transfusions in a hyperhemolytic episode are able to accelerate hemolysis [3], which was seen in our case. Jka negative blood product (compatible blood) could not increase Hb level in our patient, and his positive auto control result directed that autologous RBC destruction may occur.

It is suggested that antibodies reacting with foreign antigens on transfused cells can cause activation of complement, leading to a phenomenon known as "bystander hemolysis" [3]. Our patient RBC was negative for Jka but had anti-Jka antibodies with a DAT 4+ result for IgG and 3+ result for C3b reflecting RBCs sensitization.

Eberly et al [3] recently reported a case with hyperhemolysis without hematologic disease, that her laboratory tests results were as the same as our patient results. IVIG and steroids [8], avoiding further blood transfusion [9], plasma-to-RBC replacement [11] are different treatment of the HS. Hemolysis was a sever condition in our patient. Additional transfusion was not avoided in this case in his history, but other therapeutic options were chosen, e.g. IV steroid and IVIG without any improvement. The patient was splenectomized finally to decrease RBC destruction by macrophages. His Hb level was increased to 8.3 g/dL during 14 days. Mechery et al suggested that splenectomy could resolve the crisis of hyperhemolysis in a child with beta-thalassemia also [9].

Conclusion

Alloimmunization is a big problem in patients with chronic and multiple transfusions that may be deceased by compatible blood transfusion, transfusion avoiding, using suitable medications and finally splenectomy, due to spleen macroghages role in RBC destruction.

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Conflict of Interest

There is no conflict of interest.

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