academicJournals

Vol. 7(5), pp. 28-31, September 2016
DOI: 10.5897/JMLD2016.0132
Article Number: FAFCABC60558
ISSN 2141-2618

http://www.academicjournals.org/JMLD

Journal of Medical Laboratory and Diagnosis

Short Communication

Blood group serology analysis of one rare case of β-thalassemia major with cold autoantibodies and alloantibodies

Zhi-xin Song[1] and Yong Zou[2]*

[1]Department of Clinical Laboratory, the 3rd Hospital of Sun Yat-Sen University, Guangzhou 510630, Guangdong, China.
[2]Department of Blood Transfusion, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, Guangdong, China.

Received 30 May, 2016; Accepted 25 August, 2016

β thalassemia major is one of major public health problem in south China requiring lifelong blood transfusions for the children to stay alive. However, long-term transfusion increases the risk of the patients to produce the autoantibodies and alloantibodies due to repeated exposure to foreign antigens. In this study, we reported one rare case of β-thalassemia major with cold autoantibodies and alloantibodies. Blood group serology experiments including the absorption-elution test, irregular antibody screening, blood group identification and cross matching were used to determine the blood group serology characteristics of the patient. Results showed that high titer cold autoantibodies and alloantibodies (anti-E and anti-N) were present in serum of the ten-years-old child with β-thalassemia major. Collectively, when difficulties in blood group identification and cross matching were found in patients with β thalassemia major, a prompt and careful blood group serology analysis will help to avoid the transfusion accident due to the undetected antibodies.

Key words: Thalassemia, autoantibody, alloantibody, blood type.

INTRODUCTION

β-thalassemia (Vichinsky, 2005; Cohen et al., 2004) is a common hemoglobin disorder in Southern China especially in Guangdong and Guangxi and one of the major public health problems.In Southern China, the carrier rate of β-thalassemia is 2.54% in Guangdong (Xu et al., 2004) and 6.78% in Guangxi (Cai et al., 2002) where two provinces were thalassemia occurred most frequently. Although blood transfusions are lifesavers for thalassemia patients, long-term transfusion increase the risk of the patients to produce the autoantibodies and alloantibodies (Sadeghian et al., 2009). Alloantibodies increase the difficulty in obtaining compatible blood

*Corresponding author. E-mail: zouyong0205@163.com. Tel/Fax: +86-020-85252526.

transfusion (Cheng et al., 2012). Autoantibodies, which are antibodies directed against a patient's self-antigens, are less widely recognized as a complication of multiple transfusions but may be clinically significant. Auto-antibodies may cause autoimmune hemolytic anemias (AIHA), which will further aggravate the anemias of β-thalassemia major (Meulenbroek et al., 2015).

Autoantibodies may make it difficult to identify co-existing alloantibodies, delaying the identification of appropriately matched blood products, since most warm autoantibodies react with most donors Red Blood Cell (RBC) (Barros et al., 2010). In addition, autoantibodies can be associated with clinically significant warm autoimmune haemolytic anaemia (Bass et al., 2013). In this report, we describe the case of a child with β-thalassemia major who developed both auto- and alloantibodies following long-term transfusion.

MATERIALS AND METHODS

Case report

A ten-year-old Chinese child with β-thalassemia major, who received no regular blood transfusion, was presented to the emergency room because of high fever. The fever mainly happened at night and with no obvious cause. The temperature could reach 39.8℃, accompanied with yellowing of the eyes and skin, paleness, weary asthenia, palpitation and shortness of breath following exercise. Laboratory values on admission were as follows: White blood cell count 4.24 × 10^9/L, red blood cell count 1.01 × 10^{12}/L, haemoglobin (Hb) 2.7 g/dL, haematocrit %, and platelet count 135 × 109/L, NEUT 59.0%, total bilirubin (TBIL) 217.1 umol/L, indirect bilirubin (IBIL) 199.64 umol/L. chest X- ray report revealed bilateral pulmonary inflammation. Four unit of packed RBC were ready to be transfused to the child to ameliorate the anemia. However, there was a discrepancy between the forward and reverse typing. The direct antiglobulin test (DAT) was positive. Strong agglutination was made by autologous red cell and autoserum in the saline media at room temperature. So a blood group serology test was made to prove whether cold autoantibodies existed.

Antibody screening

Antibody screening was carried out by the tube method. The reaction was performed between patient's autoserum with screening cells (I, II, III DiaMed, Switzerland) and autologous red cells in the saline, polybrene and anti-human globulin media at room tempreture or 37°C.

The absorption-elution test

Patient's autologous red cells were washed for three times with 37℃ saline until no agglutination. 2ml packed red cells was mixed with 2ml antoserum at 4°C for 30 min, and shook every 10 min. Repeat absorption until no agglutination between the autoserum and the screening cells at the he saline media. The packed red cells were eluted at 56°C for 10 min.

Antibody identification

To further investigate to identify the antibody specificity, an 16-cell antibody identification panel (Diapanel, Switzerland) was used. The reaction between spectrum cells and patient's serum post absorption was performed in the saline, polybrene, and anti-human globulin media.

Cross matching

The patient's serum and red cells were treated by absorption and washed, respectively. Cross matching was performed by the improved polybrene kit (Zhongshan Shengke Reagent and Instrument, China) according to the manufacturer's instructions.

RESULTS

Direct Coombs test multi-specificity (Anti-IgG+Anti-C3) is monoclonal Anti-IgG4:4+; monoclonal Anti-C3:4+

Antibody screening

As shown in Table 1, all reactions showed strong agglutination in every kind of media at room temperature. However, at the saline media at 37°C, patient's autoserum only agglutinated screening cells I, and at polybrene and anti-human globulin media at 37°C, patient's autoserum agglutinated screening cells I and II), which suggested a combination of autoantibodies and alloantibodies (IgG and IgM). Further identification was needed.

The absorption-elution test

Elution liquid agglutinated all spectrum cells in the saline, polybrene and anti-human globulin media, suggested the existence of cold autoantibodies and no autoantibody specificity. The titers of cold antibodies were 1024 at 4°C and 16 at room temperature.

Blood group identification

As shown in Table 2, there was a discrepancy between the forward and reverse typing before treatment of patient's red cells and serum, while it could be identified as blood group O after treatment.

Antibody identification

As shown in Table 3, alloantibodies including anti-E and anti-N were existed in the patient's serum. In order to confirm the conclusion above, an absorption-elution test was performed between group O red cells (CCDEE, N+) and the patient's serum post cold autoantibodies absorption. The results also demonstrated the existence of allo-anti-E and allo-anti-N.

Table 1. Reaction pattern of patient's serum with screening cells and autologous cells in different media.

Screening cells	Saline		Polybrene		Anti-human globulin	
	Room temperature	37°C	Room temperature	37°C	Room temperature	37°C
1	4+	2+	4+	2+	4+	2+
2	4+	0	4+	3+	4+	3+
3	4+	0	4+	0	4+	0
Auto	4+	0	4+	0	4+	0

Table 2. Blood group identification before and after treatment of patient's red cells and serum.

	Anti-A	Anti-B	Anti-A,B	Ac	Bc	Oc	Auto c	Rh
Before	4+	4+	4+	4+	4+	4+	4+	
After	0	0	0	4+	4+	0	0	CCDee

Table 3. The reaction pattern of spectrum cells and patient's serum post cold autoantibodies absorption.

Spectrum cells	Rh-Hr								MN				P	Serum after being absorbed		
	D	C	c	E	e	f	V	Cw	M	N	S	s	P1	Saline	Polybrene	Anti-human globulin
1	+	+	0	+	+	0	0	0	0	+	0	+	+	2+	4+	4+
2	+	+	0	0	+	0	0	+	0	+	0	+	+	2+	2+	2+
3	+	0	+	+	0	0	0	0	0	+	0	+	+	4+	4+	4+
4	+	0	+	0	+	+	0	0	+	+	0	0	+	1+	1+	1+
5	0	+	+	0	+	+	0	0	+	0	+	+	+	0	0	0
6	0	0	+	+	+	+	0	0	+	+	+	+	0	2+	4+	4+
7	0	0	+	0	+	+	0	0	+	0	0	+	0	0	0	0
8	0	0	+	0	+	+	0	0	+	+	+	+	+	0	0	0
9	+	+	0	0	+	0	0	0	+	0	+	+	0	0	0	0
10	+	+	0	0	+	0	0	0	+	0	0	+	+	0	0	0
11	+	0	+	+	+	+	0	0	+	0	+	+	+	2+	4+	4+
12	0	+	+	0	+	+	0	0	+	+	+	0	+	1+	1+	1+
13	0	0	+	0	+	+	0	0	+	+	0	+	+	1+	1+	1+
14	0	0	+	0	+	+	0	0	0	+	0	0	+	2+	2+	2+
15	0	0	+	0	+	+	+	0	0	+	0	+	+	2+	3+	3+
16	0	0	+	0	+	+	0	0	+	0	+	0	+	1+	1+	1+
Auto	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0

The intension of agglutination increases from 1+ to 4+; 0 means no agglutination.

Cross matching test

Cross matching was performed according to the manufacturer's instructions. Finally, four units washed group O red cells without antigen E or antigen N from donors were transfused to the patients and showed no adverse reaction.

DISCUSSION

Thalassemia is a common inherited hemolytic disorder in southern part of China especially in Guangdong and Guangxi (Xu et al., 2004; Cai et al., 2002). Regular transfusions are essential for patients with thalassemia to maintain lives and development. However, the bodies' load of ferrum will be added up because of perennial anemia and transfusion. Complication such as respiratory infection, change of organ function and life shortening would take place due to the weakened autoimmunity (Lombardi et al., 1994; Moshtaghi-Kashanian et al., 2006; Wang et al., 2003; Vichinsky et al., 2005). On the other hand, autoantibodies, HLA antibodies and alloantibodies engender because of long-term transfusion, which may

also bring difficulties to the transfusion itself (Cheng et al., 2012; Barros et al., 2010).

Many cases of thalassemia major accompanied with autoimmune hemolytic anemia have been reported in China, which was a threat for the patients with thalassemia (Huang et al., 2005). So it is critical for patients with thalassemia to take persistent and standard transfusion treatment in the early stage. Accumulated evidence indicated that leukocyte-reduced red blood cell transfusions can lighten the adverse allogeneic reaction, reduce the production of autoantibodies, HLA antibodies and alloantibodies (Higgins et al., 1996; Miyaji et al., 2010; Sharma et al., 2010).

In this study, we reported one rare case of β-thalassemia major with cold autoantibodies and alloantibodies (anti-E and anti-N). High frequency of blood transfusion may contribute to the formation of auto- and alloantibodies. The cold autoantibodies often cause the difficulty of blood group identification and cross matching, which cover the detection of alloantibodies and increase blood transfusion risks. Therefore, when auto- and alloantibodies coexist, a prompt exclude of autoantibodies will help to avoid the transfusion accident induced by the undetected alloantibodies antibodies.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Barros MM, Blajchman MA, Bordin JO (2010) .Warm autoimmune hemolytic anemia: recent progress in understanding the immunobiology and the treatment. Transfus. Med. Rev. 4(3):195-210.

Bass GF, Tuscano ET, Tuscano JM (2013). Diagnosis and classification of autoimmune hemolytic anemia. Autoimmun Rev. 13(4-5):560-564.

Cai R, Li L, Liang X, Liu Z, Su L, Li W, Zhu Q, Mo Q, Pan L, Ouyang H, Huang L, Xu X (2002). Prevalence survey and molecular characterization of alpha and beta thalassemia in Liuzhou city of Guangxi. Zhonghua Liu Xing Bing Xue Za Zhi. 23(4):281-285.

Cheng CK, Lee CK, Lin CK (2012). Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. Transfusion 52(10):2220-2224.

Cohen AR, Galanello R, Pennell DJ, Cunningham MJ, Vichinsky E (2004). Thalassemia. Hematology Am. Soc. Hematol. Educ. Program. 2004:14-34.

Higgins VL (1996). Leukocyte-reduced blood components: patient benefits and practical applications. Oncol. Nurs. Forum. 23(4):659-667.

Huang K, Fang JP, Zhou DH, Chen C, Huang SL, Xu HG, Li Y (2005). Clinical study of ten patients with beta-thalassemia major compl icated by immune-mediated hemolytic anemia. J. Clin. Hematol. 18(1):3-6. (In Chinese)

Lombardi G, Matera R, Minervini MM, Cascavilla N, D'Arcangelo P, Carotenuto M, Di Giorgio G, Musto P (1994). Serum levels of cytokines and soluble antigens in poly transfused patients with beta-thalassemia major: relationship to immune status. Haematologica 79(5):406-412.

Meulenbroek EM, Wouters D, Zeerleder SS (2015).Lyse or not to lyse: Clinical significance of red blood cell autoantibodies. Blood Rev. 29(6):369-376.

Miyaji K, Miyamoto T, Kohira S, Itatani K, Tomoyasu T, Sato H, Ohara K (2010). The effectiveness of prestorage leukocyte-reduced red blood cell transfusion on perioperative inflammatory response with a miniaturized biocompatible bypass system. J. Thorac. Cardiovasc. Surg. 139(6):1561-1567.

Moshtaghi-Kashanian GR, Gholamhoseinian A, Hoseinimoghadam A, Rajabalian S (2006). Splenectomy changes the pattern of cytokine production in beta-thalassemic patients. Cytokine 35(5-6):253-257.

Sadeghian MH, Keramati MR, Badiei Z, Ravarian M, Ayatollahi H, Rafatpanah H, Daluei MK (2009). Alloimmunization among transfusion-dependent thalassemia patients. Asian. J. Transfus. Sci. 3(2):95-98.

Sharma AD, Sreeram G, Erb T, Grocott HP, Slaughter TF (2000). Leukocyte-reduced blood transfusions: perioperative indications, adverse effects, and cost analysis. Anesth Analg. 90(6):1315-1323.

Vichinsky E, Butensky E, Fung E, Hudes M, Theil E, Ferrell L, Williams R, Louie L, Lee PD, Harmatz P (2005). Comparison of organ dysfunction in transfused patients with SCD or beta thalassemia. Am. J. Hematol. 80(1):70-74.

Vichinsky EP (2005). Changing patterns of thalassemia worldwide. Ann. N Y Acad. Sci.1054:18-24

Wang SC, Lin KH, Chern JP, Lu MY, Jou ST, Lin DT, Lin KS (2003). Severe bacterial infection in transfusion-dependent patients with thalassemia major. Clin. Infect. Dis. 37(7):984-988.

Xu XM, Zhou YQ, Luo GX, Liao C, Zhou M, Chen PY, Lu JP, Jia SQ, Xiao GF, Shen X, Li J, Chen HP, Xia YY, Wen YX, Mo QH, Li WD, Li YY, Zhuo LW, Wang ZQ, Chen YJ, Qin CH, Zhong M (2004). The prevalence and spectrum of alpha and beta thalassaemia in Guangdong Province: implications for the future health burden and population screening. J. Clin. Pathol. 57(5):517-522.