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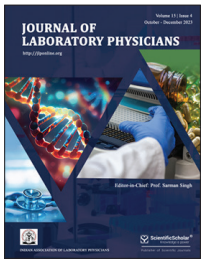

Case Report

Role of polyethylene glycol and enzymes in identifying antibody specificity in a patient with sickle cell disease

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ABSTRACT

Alloimmunization is a common problem in sickle cell disease (SCD) patients. Providing antigen-negative units in this group of patients makes it challenging for the transfusion laboratory. Antibody identification is required to decrease the incidence of hemolytic transfusion reaction in case of alloimmunization. In SCD patients, identification of antibody specificity is essential to prevent particularly delayed type transfusion reactions. This is a case report of a transfusion-dependent SCD patient whose antibody specificity could not be determined on initial antibody identification testing. A modified antibody identification test was done with polyethylene glycol (PEG) and enzyme to identify antibody specificity in this patient. Alloantibody anti-c was identified and reported to the clinician. The patient was transfused with c antigen- negative blood. This case report highlights the role of PEG and enzyme treatment in antibody identification for transfusion support in this group of highly alloimmunized patients.

Keywords: alloimmunization, polyethylene glycol, enzyme treatment

INTRODUCTION

Multitransfused patients, especially sickle cell disease (SCD) patients, possess a high chance of producing alloantibodies, which becomes a challenge in finding the specific antigen- negative packed red blood cell (PRBC) for such patients.[1] So, alloantibody identification plays a vital role in the transfusion support of such patients. But sometimes, multiple alloantibodies, autoantibodies, antibodies against high-incidence antigens, antibodies of unknown specificity, and polyagglutination are some confrontations in discovering the specific alloantibody present in the patient'sserum. The mostcommon antibody prevalence in SCD patients is against Rh blood group system antigens.[2] Antibodies against Rh antigens are clinically significant, as it causes both acute and delayed type transfusion reaction (DHTR).[3] Identifying these antibodies is essential to prevent DHTR, which is prevalent in these patients. Here, we illustrate an interesting case of a SCD patient whose antibody specificity could not be elicited on initial antibody screening and identification tests. However, with modified antibody identification techniques, such as enzyme and polyethylene glycol (PEG), anti-c antibody was identified.

CASE REPORT

A 43-year-old female patient diagnosed with SCD with frequent transfusion, that is, around one or two units of PRBCs per year, was admitted to the hematology ward. She had her last transfusion

5 months back and has no history of transfusion reactions during that time. The attending clinician of the patient had requested a blood transfusion for her because of anemia with hemoglobin of 6 g/dL. Per our institute protocol, before transfusion ABO and RhD typing, Rh, Kell phenotyping, antibody screening and identification, direct antiglobulin test (DAT), and autocontrol (AC) were performed in every SCD patient.

The ABO and RhD typing and DAT were done by column agglutination technology (Gel test, Tulip Diagnostics matrix gel card system, Goa, India). The ABO and RhD type of patient was B Rh-D positive without any discrepancy. The DAT and AC were negative in the antihuman globulin (AHG) phase. Antibody screening with Immucor 3 cell panel (Lot No: 6559), Norcross, United States, in the test tube method, showed negative reactions in the first two panels and 2þ reactions in the third panel (Table 1). Check cells were used in those red cell panels with no agglutination in the test tube AHG method. Antibody identification was performed with an Immucor 16 cell panel showing the probability of anti-c antibody. But the exact pattern of anti-c was not present in 16 cell panels as some panels showed no agglutination even if c antigen was present (Table 2). Rh, Kell phenotype was tested by test tube technique (Immucor). The patient's Rh-Kell phenotype was C-4 þ , c-negative, E-negative, e-4 þ , and Kell negative.

Modified antibody identification was performed with inhouse prepared PEG (HiMedia, Maharashtra, India) and papain (Liquipap, Tulip Diagnostic Pvt. LTD, goa, India) by test tube method. There were enhanced reactions in the panel cells with c antigen, and anti-c was confirmed (Table 2). There was no agglutination in panel 10 containing c–Eþ red cells. Another two donors containing c–Eþ red cells also had no agglutination. So anti-E antibody was ruled out. Then, the patient was transfused with c antigen-negative red cells. There was no transfusion reaction with hemoglobin 7.2 g/dL, 48 hours of follow-up. A report card with an antibody identified as anti-c was given to the patient for future transfusion. She was advised to transfuse in a blood center with a facility for antibody screening, identification, and red cell phenotype match.

DISCUSSION

In many cases, the specificity of antibodies could not be found in the antibody identification panel. Causes are usually antibodies against low prevalence antigens, non-red blood cell (RBC) antigens, or developing clinically significant antigens.[4] Multitransfused patients, especially sickle cellpatients, have the potency to produce alloantibodies. Early recognition of the antibodies and providing specific antigen- negative blood to these patients can prevent severe complications in such patients.[5] These problems can be

Table 1: Results of antibody screening panels

Antigens	D	C	c	E	E	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lub	Xga	AHG tube	CC
1	+	+	0	0	+	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	2+
R1R1																										
2	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	0	0	2+
R2R2																										
3	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	+	+	+	2+	NT
Rr																										

Abbreviations: AHG, antihuman globulin; CC, check cell; NT, not tested.

Table 2: Antibody identification panels

Antigens	D	C	c	E	E	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	AHG tube	AHG PEG	AHG papain
1 R1R1	+	+	0	0	+	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	0
2 R2R2	+	0	+	+	0	0	+	0	+	0	+	0	+	0	0	+	+	+	0	+	+	0	0	+	+	0	2+	3+
3 R2R2	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	0	+	+	2+	3+	4+
4 R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	0
5 R1R1	+	+	0	0	+	0	+	0	+	0	+	0	0	0	0	0	+	+	0	+	0	+	0	+	0	0	0	0
6 R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	0	+	0	+	+	0	0	0
7 Rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	+	0	+	+	0	2+	2+
8 Rr	0	0	+	0	+	+	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	+	+	+	+	2+	3+	4+
9 R1R1	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	+	0	0	0
10 RzR1	+	+	0	+	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	0	0	+	0	+	+	0	0	0
11 Rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	0	+	0	+	+	0	2+	2+
12 Rr	0	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	0	0	+	+	0	2+	2+
13 Rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	2+	3+	4+
14 Rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	0	0	+	0	+	0	+	+	0	2+	2+
15 Rr	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	+	+	+	+	0	+	+	1+	2+	4+
16 Rr	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	0	+	0	0	+	+	1+	3+	4+

Abbreviations: AHG, antihuman globulin; PEG, polyethylene glycol.
Notes: Check cells are tested in those panels had no agglutination reactions to validate the tube test results. There are 2þ agglutination in all previously nonagglutinated panels.

resolved with advanced techniques such as select cells, serial alloadsorption and elution techniques, and modified antibody identification tests with enzyme and PEG treatment of reagent red cells.

The PEG method accelerates the binding of the antibody to red cells by steric exclusion of water molecules in the diluent, thus concentrating the antibodies. It is more sensitive than low ionic strength solution (LISS), albumin, and saline methods.[6]

Advantage of PEG is it can detect antibodies even in LISS-negative antibody detection method.[7] However, it is inappropriate for cases such as multiple myeloma, where it causes the precipitation of proteins and produces afalse-positive reaction.[8]

Further confirmation was done with enzyme treatment with papain. Papain can destroy or weaken some RBC antigens while enhancing others. It is used in pretransfusion testing when a pattern of weak reactions fails to indicate specificity or to confirm any suspected antibody. They modify the RBC surface by removing the sialic acid residues and denaturing or removing some blood group antigens.[9] They enhance the activity of antibodies of ABO, Rh, Kidd, Lewis, I, and P blood group systems and destroy antibodies of Duffy, MNS, and Xga systems. Some other enzymes used for this purpose are ficin, bromelain, chymotrypsin, and trypsin.[10]

Most centers do not provide phenotype-matched blood units, leading to alloantibody formation in SCD patients. SCD patients are prone to DHTR. If antibody specificity is not identified, there is a chance of DHTR in case the patient is receiving transfusion in those centers without the facility of using phenotype-matched PRBC units.[1] PEG indirect antiglobulin test and enzyme-treated reagent red cells antibody identification techniques were used to detect clinically significant anti-c antibodies in our patient. Our case enlightened the importance of these techniques; otherwise, we would have missed the identification of the clinically significant anti-c antibody in this patient.

CONCLUSIONS

Antibody identification is vital in SCD patients to provide antigen-negative PRBC units. Modified antibody techniques such as PEG and enzymes should be used in case of difficulty identifying antibodies. A report card with the specificity of antibody identification and advice of phenotype-matched blood or antigen negative unit of specifically identified antibody must be given to the patient.

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Conflict of interest

None declared.

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