

Short Communication

Separation of Autologous Red Blood Cells from Specimen of the Transfused Sick Cell Disease Patients Using Hypotonic Saline

Ankita Sheladiya, Kinjal Mendapara-Dobariya, Sanmukh R Joshi

Lok Samarpan Regional
Blood Bank and Research
Center, Surat, Gujarat, India

ABSTRACT

Background: Red blood cell (RBC) antigen typing in transfused sickle cell disease (SCD) patients is a difficult task due to contamination of the transfused red cells. **Aim:** This pilot study was aimed to standardize an easy approach to separate the autologous RBCs from the transfused patient for RBC antigen typing in SCD. **Materials and Methods:** The RBCs from transfused patients with SCD were separated by hypotonic saline and tested with various antisera by standard serological methods. **Results:** Ten antisera showing negative reaction with donors' RBCs reacted positively with the patients' autologous RBCs separated by treatment with hypotonic saline. Seven antisera reacted with the donors' RBCs were nonreactive with the patients' autologous RBCs after elimination of the donor's RBCs by treatment with hypotonic saline. **Conclusion:** The hypotonic saline can be used as method to separate the autologous RBCs from the mixture with the transfused RBC in patients with SCD.

KEYWORDS: Autologous red blood cells, sickle cell disease, transfused patients

Received: January, 2017.
Accepted: February, 2017.

INTRODUCTION

The patients with sickle cell disease (SCD) often require transfusions in the face of anemia, more so during the sickle cell crisis. The transfused patients may develop alloantibodies to minor blood group antigens in 18.6% of the cases with SCD.^[1] The antibodies may pose problems in pretransfusion compatibility tests, and if inadvertently transfused an incompatible blood, the recipient may experience acute or delayed hemolytic transfusion reaction (HTR). Ideally, such patients should be typed for the common clinically significant blood group antigens beforehand, and the antigen-matched blood unit should be used in transfusion to avoid sensitization to alloantigens. In practice, however, it is not feasible to do so as the patients might already have received transfusion in the face of anemia before being diagnosed for the condition. Once transfused, it is difficult to type the patient's red cells for the minor blood group antigens due to an interference of the donor's transfused red blood cells (RBCs) until they are present in circulation. To obviate such interference of

the donor's RBCs in the transfused patient, we had used hypotonic saline to get rid of the transfused red cells from the blood specimen.^[2] The present report deals with an approach to such cases of SCD.

MATERIALS AND METHODS

A total of five patients diagnosed with SCD were included in this study. The patients' posttransfusion blood specimens, collected within 2 days in ethylenediamine tetra acetic acid, were processed for the separation of autologous RBCs. As the history of transfusion was variable among the patients and to maintain the uniformity as to the posttransfusion specimen, the current posttransfusion specimen was considered for the purpose. This has helped in a procurement of the corresponding donors' RBCs readily available from the blood bank.

Address for correspondence:

Dr. Sanmukh R Joshi, E-mail: sanmukhj@yahoo.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Sheladiya A, Mendapara-Dobariya K, Joshi SR. Separation of autologous red blood cells from specimen of the transfused sickle cell disease patients using hypotonic saline. *Glob J Transfus Med AATM* 2017;2:44-6.

Access this article online

Quick Response Code:



Website: www.gjtmonline.com

DOI: 10.4103/GJTM.GJTM_1_17

A total of 15 antisera were used which include in-house detected anti-M, -N, -P1, -Le(b), -Jk(a), -Jk(b), -S, -s, -Fy(a), -Fy(b), and -K as well as commercially purchased (Ortho Clinical Diagnostics, Mumbai, India) anti-C, -c, -e, and -E and antiglobulin reagents. The in-house detected antisera were subjected to quality control test to assess their fitness to be used in this study. The choice of the test procedures employed was as per the nature of the antibodies in the reagents, for example, saline tube test at 4°C, low-ionic strength solution, and indirect antiglobulin test through the column agglutination technique (Ortho Clinical Diagnostics, Mumbai, India) at 37°C. Appropriate control cells were run in parallel to validate the results for reactivity and specificity of the antisera used.

Separation of the autologous RBCs of the patients were carried out as reported earlier.^[2] In brief: 100 µL saline-washed packed cells was added to 40 mL of 0.3% saline and mixed intermittently for 5 min at 22°C and centrifuged at 1000 rpm for 1 min. The supernatant was discarded, and the sediment with the patient's autologous RBCs was washed with and resuspended in normal saline to get 4% concentration of RBCs for typing. For workup of each case, cell typing was performed on three sets of RBCs, namely, patient's posttransfusion sample, transfused donor unit segment, and patient's autologous RBCs separated using hypotonic saline. The antigens covered include both the clinically significant and clinically insignificant blood group systems. The data identical for the presence

or absence of antigens on the patient's autologous RBCs and those on the corresponding donor were not tabulated in the results. The in-house antisera were sourced from the patients with variable ABO groups; hence, they were used for the SCD patients who had matched.

RESULTS

The patients' posttransfusion RBC specimens showed a mixed-field agglutination pattern by all the antisera, due to the mixture of the transfused RBCs of the nonidentical antigen profile with that of the patient and vice versa. The seven sera, namely, anti-Le(b), -c, -E, -Jk(a), -s, Fy(a), and -Fy(b), showed positive reaction with the donors' RBCs, but they did not react with the patients' autologous RBCs separated by hypotonic saline. Likewise, ten sera, namely, anti-M, -N, -P1, -C, -c, -Jk(a), -Jk(b), -S, -Fy(a), and -K, showing negative reaction with the donors' RBCs, reacted with the patients' autologous RBCs separated by treatment with hypotonic saline. One of the patients with a mild delayed HTR with a positive DAT, presumably due to *in vivo* sensitization of the incompatible red cells of the transfused blood unit by the alloantibody present in the patient's plasma provided an interesting scenario. An exposure of hypotonic saline to the patient's posttransfusion red cells eliminated the antibody-coated donor's red cells; with the result, the patient's separated autologous red cells showed negative result with antiglobulin test. The results are displayed in Table 1.

Table 1: Results on cell typing of the transfused sickle cell disease patients following the elimination of the transfused red blood cells

Antisera	Number of patients	Patient's specimen (posttransfusion) [#]	Donor specimen (current transfusion)	Patient's specimen (Isolated, autologous)
Anti-M	2	+	0	+
Anti-N	2	+	0	+
Anti-P ₁	2	+	0	+
Anti-Le (b)	1	+	+	0
Anti-C	1	+	0	+
Anti-c	2	+	0	+
	1	+	+	0
Anti-E	2	+	+	0
Anti-Jk (a)	1	+	0	+
	1	+	+	0
Anti-Jk (b)	1	+	0	+
Anti-S	2	+	0	+
Anti-s	1	+	+	0
Anti-Fy (a)	1	+	0	+
	1	+	+	0
Anti-Fy (b)	1	+	+	0
Anti-K	2	+	0	+
AHG/DCT	1	+	0	0

[#]Mixed-field agglutination pattern. AHG: Anti-human globulin, DAT: Direct antiglobulin test

DISCUSSION

Multitransfused patients have greater chance to develop alloantibodies and often pose problems in finding compatible blood unit for transfusion. The patients with chronic renal failure, malignancies, beta thalassemia major, sickle cell disease (SCD), etc., are vulnerable to get immunized following transfusions. Once clinically significant antibodies are produced, they pose problems in finding compatible blood for transfusion as well as make it difficult to differentiate whether the antibody present is of alloantibody or autoantibody nature. The RBCs of the patients with sickle cells disease or thalassemia major are more resistant to osmotic insult than those from normal individual.^[3] Exploiting this property, Sheladiya *et al.*^[2] and Brown^[4] had used hypotonic saline to get rid of transfused RBCs to isolate the autologous RBCs from such transfused patients. The RBCs typing should be carried out using reagent grade antisera, for they are potent and specific. The cell typing can also be done by various approaches such as use of the reticulocytes separated by density gradients,^[5-7] or using two-color flow cytometry,^[8] or molecular typing using the DNA.^[9] However, the use of reticulocytes for the purpose may have limitations as certain blood group antigens are poorly expressed on the reticulocytes.^[10] Many of these approaches are not only expensive, but they also require specialized setup and expertise to handle the tests, and that many blood banks may not be able to afford these resources. The method described here provides an easy approach to eliminate the transfused RBCs that come in the way of cell typing in transfused patients. The method is simple, rapid, and affordable for any blood bank that deals with transfusion to such patients.

CONCLUSION

Separation of autologous RBCs from transfused SCD patients using hypotonic saline is an easy, quick, and

inexpensive approach for a resource-constrained blood bank dealing with blood supply to multitransfused patients with SCD.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Rosse WF, Gallagher D, Kinney TR, Castro O, Dosik H, Moohr J, *et al.* Transfusion and alloimmunization in sickle cell disease. The cooperative study of Sickle Cell Disease. *Blood* 1990;76:1431-7.
2. Sheladiya A, Mendapara K, Joshi SR. Isolation of autologous RBCs in transfused β -thalassemia patients using hypotonic saline. *Transfus Med* 2016;26:385-6.
3. Chow J, Phelan L, Bain BJ. Evaluation of single-tube osmotic fragility as a screening test for thalassemia. *Am J Hematol* 2005;79:198-201.
4. Brown DJ. A rapid method for harvesting autologous red cells from patients with hemoglobin S disease. *Transfusion* 1988;28:21-3.
5. Wallas CH, Tanley PC, Gorrell LP. Recovery of autologous erythrocytes in transfused patients. *Transfusion* 1980;20:332-6.
6. Branch DR, Hian AL, Carlson F, Maslow WC, Petz LD. Erythrocyte age-fractionation using a Percoll-renografin density gradient: Application to autologous red cell antigen determinations in recently transfused patients. *Am J Clin Pathol* 1983;80:453-8.
7. Brun A, Skadberg O, Hervig TA, Sandberg S. Phenotyping autologous red cells within 1 day after allogeneic blood transfusion by using immunomagnetic isolation of reticulocytes. *Transfusion* 1994;34:162-6.
8. Griffin GD, Lippert LE, Dow NS, Berger TA, Hickman MR, Salata KF. A flow cytometric method for phenotyping recipient red cells following transfusion. *Transfusion* 1994;34:233-7.
9. Castilho L, Rios M, Pellegrino J Jr., Saad S, Costa F. Blood group genotyping facilitates transfusion of beta-thalassemia patients. *J Clin Lab Anal* 2002;16:216-20.
10. Vengelen-Tyler V, Gonzalez B. Reticulocyte rich RBCs will give weak reactions with many blood typing antisera. *Transfusion* 1985;25:476.