Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr ICV= 85.10

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Comparison of two different elution methods of Removal of antibodies from red cells

Dr. Tarun Kumar[1], Dr. Seema Solanki[2], Dr. Manav Vadhera[3], Dr. Jessica Kaushal[4], Dr Neeeaj Joshi[5]

[1]Assistant professor, Department of transfusion medicine, MM Medical College and Hospital, Solan, HP
[2]Assistant professor, Department of Microbiology, MM Medical College and Hospital, Solan, HP
[3]Private Consultant, Fortis hospital, Ludhiana, Punjab
[4]MBBS, Govt. Medical College & Hospital, Amritsar, Punjab
[5]Senior resident, Department of internal medicine, MMMC & Hospital, Kumarhatti, Solan, HP

***ABSTRACT*:**
Background: In transfusion medicine it may be necessary to remove antibodies (Abs) that have sensitized red blood cells (RBCs) in vivo when evaluating a patient's positive direct antiglobulin test (DAT). Hence; the present study was undertaken for assessing and comparing two different elution methods of Removal of antibodies from red cells. **Materials & methods:** Direct antiglobulin test (DAT) and Indirect antiglobulin test (IAT) were performed using gel cards. Comparison of two different elution methods as done: Heat elution and Chloroquine diphosphate elution methods. Both the methods were performed on all DAT positive samples and their efficacy in removal of autoantibodies was compared. Sensitization of red cells Samples of red cells sensitized in vivo were obtained from patients with warm reactive autoantibodies in their sera. A total of 40 samples which were positive by gel cards, were subjected to two elution methods. All the results were recorded and analysed using SPSS software. **Results:** Mean DAT agglutination score among heat elution group and chloroquine elution group at 1+ was 2.23 and 2.84 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 2+ was 4.84 and 4.86 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 3+ was 6.86 and 7.39 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 4+ was 8.65 and 9.81 respectively. Significant results were obtained while comparing DAT Agglutination score before and after heat elution method and chloroquine elution methods. **Conclusion:** An elution method, which requires small amount of red cells, provides quick and reliable results with little damage to the red cell antigens should be selected in patients with DAT positivity.
Key words: Antibodies, Red cells

Received: 10 January, 2021 Accepted: 18 January, 2021

Corresponding author: Dr Neeeaj Joshi, Senior resident, Department of internal medicine, MMMC & Hospital, Kumarhatti, Solan H.P (173229), Email ID: joshineeraj941@gmail.com

This article may be cited as: Kumar T, Solanki S, Vadhera M, Kaushal J, Joshi N. Comparison of two different elution methods of Removal of antibodies from red cells. J Adv Med Dent Scie Res 2021;9(3): 153-155.

INTRODUCTION

In transfusion medicine it may be necessary to remove antibodies (Abs) that have sensitized red blood cells (RBCs) in vivo when evaluating a patient's positive direct antiglobulin test (DAT). There are many reasons why patients can present with a positive DAT and these include haemolytic transfusion reactions, autoimmune haemolytic anaemia, drug-induced haemolysis, and haemolytic disease of the newborn (HDN). The identification of Abs that cause positive DATs can aid in patient diagnosis and treatment. In order to identify the attached Abs it is necessary to remove and test them against a panel of RBCs of known phenotypes.[1- 3]

Saline reactive antisera, chemically modified antisera and IgM monoclonal antibodies are available for some of the red cell antigens but; antigens detected by indirect antiglobulin test are difficult to phenotype. It is therefore necessary to remove antibodies from in vivo sensitized red

cells to phenotype them. Various elution procedures are used for dissociating antibodies from red cells.[4- 6] Hence; the present study was undertaken for assessing and comparing two different elution methods of Removal of antibodies from red cells.

MATERIALS & METHODS

The present study was conducted for assessing and comparing two different elution methods of Removal of antibodies from red cells. Data of patient which sent their samples for serological evaluation of autoimmune hemolysis were enrolled. Direct antiglobulin test (DAT) and Indirect antiglobulin test (IAT) were performed using gel cards. Comparison of two different elution methods as done: Heat elution and Chloroquine diphosphate elution methods. Both the methods were performed on all DAT positive samples and their efficacy in removal of autoantibodies was compared. Sensitization of red cells Samples of red cells sensitized in vivo were obtained from patients with warm reactive autoantibodies in their sera. A total of 40 samples which were positive by gel cards, were subjected to two elution methods. All the results were recorded and analysed using SPSS software. Chi-square test was used for evaluation of level of significance.

RESULTS

A total of 40 samples which were positive by gel cards, were subjected to two elution methods. Comparison of two different elution methods as done: Heat elution and Chloroquine diphosphate elution methods. Mean DAT agglutination score among heat elution group and chloroquine elution group at 1+ was 2.23 and 2.84 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 2+ was 4.84 and 4.86 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 3+ was 6.86 and 7.39 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 4+ was 8.65 and 9.81 respectively. Significant results were obtained while comparing DAT Agglutination score before and after heat elution method and chloroquine elution methods.

Table 1: Comparison of DAT Agglutination score before and after heat elution method and chloroquine elution methods

DAT	Heat elution method	Chloroquine elution methods	p-value
Wk + ve	0.53	0.79	0.00*
1 +	2.23	2.84	0.45
2 +	4.84	4.86	0.36
3 +	6.86	7.39	0.01*
4 +	8.65	9.81	0.02*
Total	4.78	5.35	-

*: Significant

DISCUSSION

Red blood cells (RBCs) can be removed from the bloodstream at an accelerated rate by antibody-mediated mechanisms in a number of immunologic conditions including the following: 1) autoimmune hemolytic anemias, 2) transfusion of incompatible allogeneic RBCs into a recipient whose blood contains a corresponding alloantibody, 3) transfusion of passive antibodies into a recipient whose RBCs express the cognate antigen, 4) transplacental transfer of maternal allogeneic RBC antibodies into a fetus with RBCs expressing the cognate antigen resulting in alloimmune hemolytic disease of the fetus and newborn, and 5) posttransplantation transfer of viable donor lymphocytes capable of producing antibodies directed against recipient RBCs (e.g., group O donor into group A recipient).[6- 9] Hence; the present study was undertaken for assessing and comparing two different elution methods of Removal of antibodies from red cells.

In the present study, A total of 40 samples which were positive by gel cards, were subjected to two elution methods. Comparison of two different elution methods as done: Heat elution and Chloroquine diphosphate elution methods. Mean DAT agglutination score among heat elution group and chloroquine elution group at 1+ was 2.23 and 2.84 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 2+ was 4.84 and 4.86 respectively. Mean DAT agglutination score among heat elution group and chloroquine elution group at 3+ was 6.86 and 7.39 respectively. Rahul Katharia et al studied the efficacy of various elution methods in removing the antibodies coating the red cells and their impact on different blood group antigen activity. Patient samples sent for serological evaluation of autoimmune hemolysis were included in the study. DAT and Indirect antiglobulin test (IAT) were performed using gel cards (ID system, DiaMed Switzerland). Antibody coated red cells, either by in-vivo or in-vitro sensitization, were used to assess the outcome of three elution methods. Out of 93 DAT positive samples already sensitized in vivo, 28 (30 %) samples became DAT negative post elution using either of three methods, while 36 (38.8%) showed reduction in strength of reaction, whereas in 29 (31.2%) there was no change in strength of reaction. Similarly, out of the 17 samples prepared by in vitro sensitization, 12 samples became completely negative after glycine-HCl/EDTA elution, 9 and 5 samples became negative after heat elution and chloroquine diphosphate elution methods, respectively. On comparative analysis glycine-HCl/EDTA elution method was better than the other two methods and can be used for eluting immunoglobulins from intact red cells.[10]

N Burin des Roziers compared methods of removing IgG antibodies from intact red cells. Antibodies coating red cells that were sensitized in vivo (warm-reactive autoantibodies: 8 patients) or in vitro (42 alloantibodies) were eluted by using glycine-HCl and EDTA (acid/

EDTA), heat (56 degrees C, 10 min), or chloroquine method. Acid/EDTA elution gave the best results, reducing DAT positivity to microscopic levels or rendering the DAT negative in 48 of 50 instances, whereas 4 samples remained resistant to heat elution and 24 to chloroquine. Standard DAT agglutination scores demonstrated that both acid/EDTA and heat elution were superior to the chloroquine method ($p < 0.0001$). With the gel low-ionic-strength saline indirect antiglobulin test, acid/ EDTA was superior to heat ($p < 0.001$). Overall, acid/ EDTA elution dissociated more antibodies than heat ($p < 0.0001$), especially for Kell system (K, k, Kpa, Kpb) alloantibodies. Common red cell antigens, other than Kell system antigens, were unaffected by acid/EDTA elution. In contrast, the expression of most blood group antigens was diminished after heat elution. However, it was possible to type red cell antigens by using gel low-ionic-strength saline indirect antiglobulin tests or tube agglutination methods. Although heat elution may be used on a limited basis, the acid/EDTA method appears to be the procedure of choice for typing red cell coated with warm-reactive IgG alloantibodies or autoantibodies.[11]

In the present study, mean DAT agglutination score among heat elution group and chloroquine elution group at 4+ was 8.65 and 9.81 respectively. Significant results were obtained while comparing DAT Agglutination score before and after heat elution method and chloroquine elution methods. Mock DM et al documented the ability of a method using biotin-labeled RBCs (BioRBCs) to measure RBC survival (RCS) shortened by coating with a highly purified monomeric immunoglobulin G antibody to D antigen. Autologous RBCs from 10 healthy D+ subjects were labeled with either biotin or 51Cr (reference method), coated (opsonized) either lightly ($n = 4$) or heavily ($n = 6$) with anti-D, and transfused. RCS was determined for BioRBCs and for 51Cr independently as assessed by three variables: 1) posttransfusion recovery at 24 hours (PTR24) for short-term RCS; 2) time to 50% decrease of the label (T50), and 3) mean potential life span (MPL) for long-term RCS. BioRBCs tracked both normal and shortened RCS accurately relative to 51Cr. For lightly coated RBCs, mean PTR24, T50, and MPL results were not different between BioRBCs and 51Cr. For heavily coated RBCs, both short-term and long-term RCS were shortened by approximately 17 and 50%, respectively. Mean PTR24 by BioRBCs (84 ± 18%) was not different from 51Cr (81 ± 10%); mean T50 by BioRBCs (23 ± 17 days) was not different from 51Cr (22 ± 18 days). RCS shortened by coating with anti-D can be accurately measured by BioRBCs.[12]

CONCLUSION

An elution method, which requires small amount of red cells, provides quick and reliable results with little damage to the red cell antigens should be selected in patients with DAT positivity.

REFERENCES

1. Franco RS, Lohmann J, Silverstein EB, Mayfield-Pratt G, Rucknagel DL, Joiner CH, Weiner M. The use of biotinylated RBC to determine the in vivo survival characteristics of normal and sickle cells. Blood. 1996;88:11A.
2. Russo V, Barker-Gear R, Gates R, Franco R. Studies with biotinylated RBC: (1) use of flow cytometry to determine posttransfusion survival and (2) isolation using streptavidin conjugated magnetic beads. In: Magnani M, DeLoach JR, editors. The use of resealed erythrocytes as carriers and bioreactors. Plenum Press; New York: 1992. pp. 101–7.
3. Ellis JP, Sokol RJ: Detection of IgM autoantibodies in eluates from red blood cells. Clin Lab Haematol 1990; 12:9–15
4. Fiorin F, Cozzi MR, Pradella P, Steffan A, Potenza R, De Angelis V: An original method to study autoantibody specificity in haemoglobin stained eluates by the column agglutination techniques. Clin Lab Haematol 1997; 19:209–211
5. Cordle DG, Strauss RG, Lankford GL, Mock DM. Antibodies provoked by the transfusion of biotin-labeled red cells. Transfusion. 1999;39:1065–9.
6. Massuet L, Martin C, Ribera A: Antibody elution from red blood cells by chloroform and trichloroethylene. Transfusion 1982; 22:359–361.
7. Mock DM, Lankford GL, Burmeister LF, Strauss RG. Circulating red cell volume and red cell survival can be accurately determined in sheep using the [14C]cyanate label. Pediatr Res. 1997;41:916–21.
8. Mollison P, Crome P, Hughes-Jones N, Rochna E. Rate of removal from the circulation of red cells sensitized with different amounts of antibody. Transfusion. 1965;11:461–70
9. Franco RS, Lohmann J, Silbertein EB, Mayfiled-Pratt G, Palascak M, Nemeth TA, Joiner CH, Weiner M, Rucknagel DL. Time-dependent changes in the density and hemoglobin F content of biotin-labeled sickle cells. J Clin Invest. 1998;101:2730–40.
10. Rahul Katharia 1, Rajendra K Chaudhary. Removal of antibodies from red cells: Comparison of three elution methods. Asian J Transfus Sci. 2013 Jan;7(1):29-32.
11. N Burin des Roziers 1, S Squalli. Removing IgG antibodies from intact red cells: comparison of acid and EDTA, heat, and chloroquine elution methods. Transfusion. 1997 May;37(5):497-501.
12. Mock DM, Lankford GL, Matthews NI, et al. Accelerated removal of antibody-coated red blood cells from the circulation is accurately tracked by a biotin label. Transfusion. 2012;52(5):1097-1105.