International Journal of Research in Medical Sciences
Mishra D et al. Int J Res Med Sci. 2018 Jul;6(7):2348-2353
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20182815

Original Research Article

Resolving blood group discrepancy in patients of tertiary care centre in Odisha, India

Debasish Mishra[1]*, Pankaj Parida[2], Smita Mahapatra[2], Binay Bhusan Sahoo[3]

[1]Department of Transfusion Medicine, AIIMS, Bhubaneswar, Odisha, India
[2]Department of Transfusion Medicine, SCB MCH, Cuttack, Odisha, India
[3]Odisha Blood Bank, Baripada, Odisha, India

Received: 23 April 2018
Revised: 30 April 2018
Accepted: 02 June 2018

***Correspondence:**
Dr. Debasish Mishra,
E-mail: dr.debasish01@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Blood grouping consists of both forward grouping; reverse grouping and both procedures should agree with each other. A blood group discrepancy exists when results of red cell testing do not agree with serum testing, usually due to unexpected negative or positive results in either forward or reverse typing. ABO and Rh blood group discrepancy is associated with incompatible transfusion reaction. Blood group discrepancy should be resolved before transfusion and blood group to be properly labeled to prevent transfusion reaction.
Methods: A prospective study was carried in SCB blood bank which is under the Department of Transfusion Medicine, SCB Medical College and Hospital, Cuttack, Odisha from January 2015 to October-2016. Total 25,559 blood samples of patients were included in the study and hemolysed samples excluded. The ABO and Rh D typing was done by tube technique using monoclonal IgM (Tulip Diagnostic P Ltd.) Anti-A, Anti-B, Anti-D and pooled A, B and O cell.
Results: A total of 25,559 blood group testing were done where we found 57 blood group discrepancies with overall frequency was 0.22%. Out of 57 discrepancies we were found 20 (35.09%) cases of technical error and 37 (64.91%) cases of sample related error. Among these sample related problems, we found weak/missing antibody, weak antigen expression, rouleaux, cold autoantibodies, cold alloantibodies, Bombay phenotype with the frequency of 13.51%, 2.70%, 2.70%, 54.06%, 8.11%, 18.92% respectively.
Conclusions: Mistyping either a donor or a recipient can lead to transfusion with ABO-incompatible blood, which can result in severe hemolysis and may even result in the death of the recipient. Any discrepancy between forward and reverse blood grouping methods should be resolved before transfusion of blood components.

Keywords: Blood group discrepancy, Incompatible transfusion, Technical error

INTRODUCTION

ABO Blood Group System was discovered by Karl Landsteiner in 1900, which was the most important Blood Group System in Transfusion Medicine.[1] The ABO system consists of three antigens A, B and H. On the basis of these antigens, there are four blood group phenotypes A, B, AB and O. An important feature of ABO system is the regular presence of naturally occurring IgM antibodies such as anti-A, anti-B with absence of corresponding antigens.[2] Blood grouping consists of both forward grouping; reverse grouping and both procedures should agree with each other.[3] A blood

group discrepancy exists when results of red cell testing do not agree with serum testing, usually due to unexpected negative or positive results in either forward or reverse typing. ABO and Rh blood group discrepancy is associated with incompatible transfusion reaction.[4,5] Blood group discrepancy should be resolved before transfusion and blood group to be properly labelled to prevent transfusion reaction.[6]

Blood group discrepancy may be due to technical errors and sample related problems. Common sources of technical errors are because of incorrect or inadequate identification of blood specimen, mix-up of samples, failure to add reagents, contaminated reagents etc. Sample related problem was divided further into two groups: unexpected reaction in cell or serum grouping.

Discrepancy may be arbitrarily divided into four major categories: group I, group II, group III and group IV. Group I discrepancies are associated with unexpected reaction in reverse grouping due to weakly reacting or missing antibodies. Group II discrepancies are associated with unexpected reaction in the forward grouping due to missing or weakly reacting antigens. But in group III and IV discrepancies there is problem in both forward and reverse grouping due to plasma protein and cold autoantibodies or unexpected issoagglutinins respectively.

METHODS

A prospective study was carried in SCB blood bank which is under the Department of Transfusion Medicine, SCB Medical College and Hospital, Cuttack, Odisha from January 2015 to October-2016. Total 25,559 blood samples of patients were included in the study and hemolysed samples excluded. Three mililitres of venous blood samples was collected in each plain and EDTA vacuitainer vials. The EDTA samples were used to prepare cell suspension for forward grouping. The serum samples were used for reverse grouping. All blood samples were analyzed immediately or stored between 2-6° C to prevent deterioration of weak antibodies or contamination. The ABO and Rh D typing was done by Tube technique using Monoclonal IgM (Tulip Diagnostic P Ltd.) Anti-A, Anti-B, Anti-D and pooled A, B and O cell.

Forward grouping was done by adding one drop of anti-A, anti-B, anti-D with one drop of 5% red cell suspension of patient with three labelled tube as anti-A, anti-B, anti-D. After 5-10 minutes incubation at room temperature, centrifuged the tubes at 1000 rpm for 1 minute and result was recorded. Reverse grouping was done by adding two drops of patient serum with one drop of 5% red cell suspension pooled A, B, O cell in pre-labelled three tubes as A, B and O. Then same procedure repeated as forward grouping. The results of the grouping were interpreted as per the grading system (4+ to w+) on the basis of agglutination reactions.

Blood grouping discrepancy was resolved by repeating grouping on a fresh sample to rule out technical error. A new sample for testing should be requested if discrepant results not resolved with repeat testing on same sample. Patient's medical diagnosis, historical blood group, transfusion and transplantation history were reviewed that interfered with blood grouping. A logical approach to solve sample related discrepancy is to select the side of the testing (red cell or plasma) and focusing the problem. Weak or missing antigen expression resolved by, finding out the patient's diagnosis and transfusion history. Repeating the red cell testing with extended incubation times and including monoclonal blend anti-A, B. By extending incubation time enhancement of antigen-antibody interaction occurs.

Cold autoantibodies react against all adult cells including Screening cells, A_1 and B cells and auto logus cells. An auto control is used to differentiate between cold autoantibodies from cold alloantibodies. If the auto control is positive, the reactions observed with the A1 and B cells and screening cells are probably the result of autoantibodies. Strategy to distinguish between cold autoantibodies or cold alloantibodies are, by testing the patient's serum with screening cells and an auto control at room temperature. If an alloantibody is detected, antibody identification techniques can be performed. In case, if an autoantibody is detected, special techniques to identify the antibody (mini-cold panel) and remove antibody reactivity (prewarming techniques) can be used.

Discrepancy due to rouleaux resolved by washing the red cells and repeats both forward and reverse grouping. Saline replacement technique used to distinguish true agglutination from rouleaux. Bombay phenotype does not react with anti-H lectin (Ulex europaeus) where as normal O cell individual react.

RESULTS

A total of 25,559 blood group testing's were done where we found 57 blood group discrepancies with overall frequency was 0.22%. Out of 57 discrepancies we were found 20 (35.09%) cases of technical error and 37 (64.91%) cases of sample related error. Sample related error was the most common cause of all discrepancy. (Table 1) Among these sample related problems, we found weak/missing antibody, weak antigen expression, rouleaux, cold autoantibodies, cold alloantibodies, Bombay phenotype with the frequency of 13.51%, 2.70%, 2.70%, 54.06%, 8.11%, 18.92% respectively. Cold autoantibody was the common cause of sample related blood group discrepancy.

Table 1: Distribution of types of discrepancy (N=57).

Type of discrepancy	Number	Frequency (%)
Technical error	20	35.09
Sample related error	37	64.91

ABO discrepancies were divided in to four major categories: group I, group II, group III, group IV. Group I were associated with unexpected reaction in reverse grouping due to weakly reacting or missing antibodies, which constituted 8.77% in our study. Group II discrepancies were observed to be 1.75% which showed unexpected reaction in the forward grouping due to weakly reacting or missing antigens. The frequency of group III discrepancies was found to be 1.75% mostly due to rouleaux. The miscellaneous problems such as cold autoantibodies, cold alloantibodies and Bombay phenotypes were under group IV which constituted 52.63%. The malignancy and autoagglutinins/excess protein coating red cells were the main reasons in red cell testing discrepancies in our study. The frequency of malignancy which interfered in weak red cell reactivity was 1.7%. The autoagglutinin (36.8%) was most common red cell discripancies mostly due to extra red cell activity.

Cold autoantibodies (35.1%) caused maximum discrepancies as extra reaction in serum grouping. Among cold alloantibodies, the discrepancy was identified due to anti-P1 was in 1.7% cases, where as in 3.5% cases we could not identified the alloantibody. The discrepancy in serum typing due to excess serum protein was 1.7%. Hypogammaglobulemia and elderly were two main causes of weak or missing serum reactivity which interfered with serum testing in our study. Discrepancy seen hypogammaglobulemia and elderly patient was 3.4% and 5.1% respectively.

DISCUSSION

A total 25,559 blood group testing of the patients was done from January-2015 to December-2016. Fifty-seven cases of discrepancies found between forward and reverse method were evaluated to determine the etiology and main cause of discrepancies. The present study observed that the incidence of blood group discrepancies was 0.22% which was comparable to Heo et al, from Korea who found it to 0.14%.[7] A similar study was conducted in Korea by Kim et al and in Saudi Arabia by Bashawri et al, with an incidence of 0.08% and 0.05% respectively[8,9] (Table 2).

Table 2: Comparison of overall frequency of discrepancy of other published study.

Study	Total no. of blood test	Total discrepancy	Overall frequency
Present study	25,559	57	0.22
Bashawri et al[9]	549,229	261	0.05
Kim et al[8]	93800	82	0.08
Heo MS et al[7]	-	55	0.14

In our study, the most common type of discrepancy observed was sample related error (64.91%). The other type's discrepancy was technical error with a frequency of 35.09 %. Technical error included incorrect recording of results, failure to add reagents and contaminated reagents, mislabelling, patients with same medical number, patients with two or more different medical numbers and change of medical record number were the common causes.

In our study, the mislabelling incidence was the highest (5.26%) among the errors in misidentification of samples. The frequencies of other causes of misidentification of sample errors were found to be 1.75%, 3.5%, 3.5% respectively (Table 3). Our study was comparable with Bashwari et al, study who found Mislabelling (14.6%) was the main cause of error in misidentification of samples.[9] Mislabelling was due to mixing-up sample collection or putting labels on the tubes. This error can be avoided by using a hand held electronic system to generate pretransfusion sample labels from data on the patient's wrist band at the bedside or by collecting and labelling the sample by two separate phlebotomists independently.[10,11]

Table 3: Frequency of misidentification of samples among total discrepancies.

Causes misidentification of samples	Present study (n=57) (%)	Bashwari layla et al[9] (n=261) (%)
Mislabelling	3 (5.26)	38 (14.6)
Patients with same medical number	1 (1.75)	3 (1.1)
Patients with two or more different medical number	2 (3.5)	5 (1.9)
Change of medical record number	2 (3.5)	2 (0.8)

Table 4: Different categories of sample related problems.

Group	Cause	Number	Frequency (%)
I	Weak/missing antibody	5	8.77
II	Weak/missing antigen	1	1.75
III	Rouleaux	1	1.75
IV	Miscellaneous problems	30	52.63

ABO discrepancies were divided in to four major categories: group I, group II, group III, group IV. Group I were associated with unexpected reaction in reverse grouping due to weakly reacting or missing antibodies, which constituted 8.77% in our study. Group II discrepancies were observed to be 1.75% which showed unexpected reaction in the forward grouping due to weakly reacting or missing antigens. The frequency of group III discrepancies was found to be 1.75% mostly due to rouleaux. The Miscellaneous problems such as cold autoantibodies, cold alloantibodies and Bombay phenotypes were under group IV which constituted 52.63% (Table 4).

Weak/missing antibody, cold alloantibody and Bombay phenotype showed group discrepancy in reverse typing in our study with a frequency of 13.51%, 8.11%, and 18.92% respectively. The cold autoantibodies (54.06%) was observed as the most common cause among sample related problems in our study which showed discrepancy both in forward and reverse type.

In our study we found elderly patients with age ranging from 70 years to 107 years with mean age of 92years which is comparable to the Esmali et al, study who found the mean age of elderly patient was 93 years age of the patients ranged from 88 years to 113years.[12] Weak or missing antibodies were most common in 4 cases of group O elderly patient which is comparable with Esmali et al (28 cases). Elderly patients, especially patients older than 65 years of age, have low titers of anti-A or anti-B and may have weak-reacting or missing expected antibodies. Therefore, the result of back type or reverse grouping may not be reliable in elderly patients, and forward grouping method is recommended for determining ABO blood group in elderly patients. The best way to resolve the discrepancy in age related weak or missing antibody group, is enhancing the reaction in reverse method by incubation the patients' serum with the reagent cells at room temperature for approximately 15-30 minutes. If it was not resolved, then the serum testing was repeated by incubating lowering at 4°C.[13]

This discrepancy occur either due weak or missing of red cell antigen or due to extra red cell antigen during agglutination. In our study, the cause of weak or missing antigen was malignancy with a frequency of 1.7% and the cause of extra red activity was autoagglutinin with a frequency of 35.1%. Heo et al, reported that the frequency of malignancy and autoagglutinin which interfered in red cell testing were 5.5% and 3.5% respectively (Table 5).[7] We found one case of group discrepancy in group-A individual diagnosed as acute leukemia due to weak antigen expression. To resolve this, we repeated the red cell testing with monoclonal anti-AB, anti- A1 lectin and anti-H lectin to know whether any subgroup was present or not. Poly clonal anti-A was used to adsorbed the A antigen on the surface of Red cell. The anti-A was then removed from the antigen by Elution method. The eluates were tested against three consecutive set the A cell, B cell and O cell. The result was interpreted as weak A group when it showed positive reaction with three set of A cell and negative reaction with three sets of B cell and O cell.[14]

Table 5: Type of discrepancy due to red cell testing among total discrepancies.

Discrepancy in red cell testing	Cause	Present study number (%)	Heo min-seok et al (%)[7]
Weak/missing red cell reactivity	Malignancy	1 (1.7)	3 (5.5)
Extra red cell activity	Autoagglutinins/excess protein coating red cells	21 (36.8)	2 (3.5)

Rouleaux is a stacking of erythrocytes that adhere in a coin like fashion, giving the appearance of agglutination which is caused by protein or plasma abnormalities.[15] Both forward and reverse grouping abnormalities were encountered in Rouleaux formation which was found to be 2.70% in the present study. In the cases of Rouleaux formation, the discrepancy was resolved by washing of red blood cells used in forward grouping with saline, and saline replacement techniques was used to obtain a valid reverse grouping.[14] The discrepancy in red cell testing is otherwise known as forward grouping discrepancy.

The causes of serum testing (Reverse type) discrepancies are as follows: cold alloantibody, cold autoantibody, Excess serum protein, infection and weak/missing serum reactivity. Unexpected alloantibodies mostly cold alloantiboies in the patient serum caused a discrepancy in reverse grouping. Among cold alloagglutinins anti-P was found in 1.7% cases where as in our study 3.5% cases we could not identified antibodies. A similar result was found by Heo et al, anti-P1 and unidentified antibody with a frequency 3.6% and 1.8% respectively.[7] Elevated protein level also interfered in serum testing by forming pseudo agglutination. We observed 1.7% cases of discrepancy due to excess serum protein.

The age of patients having cold agglutinin ranged from 9 to 75years with a mean age 26years. This contradicts the study of Esmaili et al, who found the mean age of cold agglutinin was 63 years which ranged from 55years to

65years.[12] Out of 23 cold agglutinin (both autoantibodies and alloantibodies), 17 patients were group-O, 4 were group-B and rest 2 were group-A individuals. The similar study was conducted by Esmali et al, who reported those 2 cases each from group-O and group-B and one case each from group-A and group-AB.[12] In the cases of cold agglutinins, the discrepancy was resolved by washing of red blood cells used in forward grouping with warm (37°c) saline to obtaining immunoglobulin-free red cells.

Patient's history and diagnosis play important role in of ABO grouping because the disease may cause grouping discrepancies. AIHA (15.78%), SLE (8.77%), anemia (10.52%), and tuberculosis (1.75%), pneumonia (1.75%) was associated with cold autoantibodies which caused blood group discrepancies both in serum testing and red cell testing. Multiple myeloma (1.75%) causes rouleaux formation which interferes with both forward and reverse grouping. Pregnancy is a physiological condition where we found discrepancies in forward grouping. Among cold alloantibodies cases, the discrepancies were noticed in 5.26% of Sickle cell disease patients and 3.5% of Thalassemia patients. The reverse type of discrepancies was observed in carcinoma of gall bladder, Lymphoma and Hypoproteinemia with a frequency of 1.75%, 3.5% and 1.75% respectively.

Bum et al, reported two cases with hepatocellular and gallbladder carcinoma where the discrepancy was with red cell type as O group and reverse typing showing anti-A only.[16] Similar case was observed in our study with a patient of gallbladder carcinoma whose forward type was O group but reverse typing was show anti-B only. In our study, we found a case of acute leukemia that showed discrepancy due to weak A antigen expression in red cell testing. Similar report was published by Picker et al, showing that 49 women with blood group A and associated with acute myeloid leukaemia whose A antigen was undetectable even by tube-spin method.[17]

CONCLUSION

ABO blood group is the most important blood group system in Transfusion Medicine and blood banking. A Blood grouping should include both forward (cell type) and reverse (serum type) methods, and the results of two methods should match and agree with each other. ABO and Rh grouping are the most important pre-transfusion tests. Mistyping either a donor or a recipient can lead to transfusion with ABO-incompatible blood, which can result in severe hemolysis and may even result in the death of the recipient. Any discrepancy between forward and reverse blood grouping methods should be resolved before transfusion of blood components. ABO discrepancies could result from errors made by hospital staff during phlebotomy and collection of specimens. In our study we found 5.26% of cases due to mislabelling. This can be prevented by proper collection and labelling of specimens during and after specimen collection to avoid any fatal complications. Technical errors are also causes of blood group discrepancy and were observed 35.09% in present study. To avoid such errors, the technical staffs should be educated through proper training and implementation of advance technology such as automation in grouping.

Sometimes we encounter weak/missing antigens that were found 2.7% in our study. ABO subgroup was one of the cause of weak/missing antigen but was not seen our study. Repeated testing and investigations such as saliva study and molecular technology for ABO subgroup is very important. It is important to recognize discrepant results and resolve them. Correct blood typing and labeling of an individual are essential to prevent ABO incompatibility. It is always important to note the strength of reaction as weaker reactions usually invite suspicion. All the patients were personally informed about their group and given a special blood group card clarifying their recipient status to prevent incompatible transfusion reaction in case of weak subgroups.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Sazama K. Reports of 355 Transfusion-associated Deaths: 1976 through 1985. Transfusion. 1990;30:583-90.
2. Hakomori S. Blood Group ABH and Ii antigens of human erythrocytes: chemistry, polymorphism, and their devlopmental change. Semin Hematol. 1981;18:39-62.
3. Makroo RN. ABO Blood group system. Compendium of Transfusion Medicine. 1st edition. New Delhi, Alp Printers. 1999;28-32.
4. Cangialosi TJ, Riolo ML, Owens SE, Dykhouse VJ, Moffitt AH, Grubb JE, Greco PM, English JD, James RD. The ABO discrepancy index: a measure of case complexity. Am J Ortho Dento Orthoped. 2004 Mar 1;125(3):270-8.
5. Khan MN, khan TA, Ahmed Z. Discrepancy in ABO Blood grouping. J Coll Physicians Surg Pak. 2013;23:590-592.
6. Denise M. Harmening, Deborah Firestone. ABO blood groups, Modern Blood Banking and Transfusion Practices. 3rd edition. New Delhi, Jaypee Brothers Medical publishers (p) Ltd. 1998;89-101.
7. Heo MS, Cho D, Park HR, Shin MG, Shin JH, Suh SP, Ryang DW. Analysis of ABO Blood Discrepancies and Transfusion Experiences in Chonnam National University Hospital. Korean J Blood Transfusion. 2013 Dec 1;24(3):222-32.
8. Esmaili HA, Taghipour H, Talghini S, Mohammadhoseyni B. An Analysis of Discrepancies between forward and reverse ABO

blood grouping. Res J Pharma. Bio Chem Sci. 2014;5(3):1360-6.
9. Qutub H, ABIM A. Analysis of ABO Discrepancies Occurring at a University Hospital, Al-khobar, Saudi Arabia. Bahrain Medical Bulletin. 2009 Dec;31(4).
10. AuBuchon JP. How I minimize mistransfusion risk in my hospital. Transfusion. 2006;46:1085-9.
11. Jensen NJ, Crosson JT. An automated system for bedside verification of the match between patient identification and blood unit identification. Transfusion 1996;36:216-21.
12. Esmaili HA, Taghipour H, Talghini S, Mohammadhoseyni B. An analysis of discrepancies between forward and reverse ABO blood grouping. Res J Pharma. Bio Chem Sci. 2014;5(3):1360-6.
13. Brecher ME. Technical Manual, 17th edn. Bethesda, MD: American Association of Blood Bank. 2011:879.
14. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montogomery Scientific Publications. 1998:1135.
15. Howard PR. In: Blaney KD, Howard PR. Concepts of immunohematology. St Louis: Moshy. 2000:44:98-9.
16. Suh IB, Chang EA, Kim HJ, Kang CD, Lee SJ, Kim TS, et al. Two cases of ABO discrepancy due to hypogammaglobulinemia. Korean J Blood Transfusion. 2003 Dec 1;14(2):240-5.
17. Picker SM, Radojska S, Rojo J, Scheid C, Gathof BS. Transfusion Med Hemother. 2004;31:181-3.

Cite this article as: Mishra D, Parida P, Mahapatra S, Sahoo BB. Resolving blood group discrepancy in patients of tertiary care centre in Odisha, India. Int J Res Med Sci 2018;6:2348-53.