

ORIGINAL ARTICLE

ABO BLOOD GROUP DISCREPANCIES AMONG THE RECIPIENTS IN A TERTIARY CARE CENTRE

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Abstract:

Background: ABO Blood Group discrepancy means the difference between forward and reverse grouping due to an unexpected extra reaction or a missing reaction. This is one of the major reasons for a transfusion reaction. The aim of this study was to analyze ABO blood group discrepancies in an algorithmic manner and find out the risk factors among the recipients. **Methods:** This cross-sectional observational study was carried out among 200 patients in the Department of Transfusion Medicine, BSMMU, Dhaka, for a period of 6 months. Both forward and reverse blood grouping were done among the recipients' sample and risk factors related to ABO grouping discrepancies was evaluated. **Results:** Presence of ABO discrepancies were found in 5.5% blood recipients (n=11). Among them the major risk factors were transfusion dependent thalassemia (TDT), mismatched transfusion, autoimmune hemolytic anemia (AIHA) and multiple myeloma (2 patients in each category, 1% each). Furthermore, 83.0% recipients were having blood transfusion >5 times. **Conclusion:** Despite all precautions ABO discrepancies still exist in transfusion sectors. Conditions where chance of ABO discrepancy is high needs extra precautions before ABO grouping to ensure safe blood transfusion.

Key words: Blood Group Discrepancy, ABO Incompatibility, Forward and Reverse Blood Grouping, Pre-transfusion testing.

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Introduction:

The ABO system, discovered by Landsteiner (1900), is crucial among all blood group systems as far as the blood transfusion is concerned. The basic of ABO grouping system depends upon the presence of antibodies in their serum against the antigen that are absent from their red blood cell. In the ABO system there are three major alleles, A, B and O, any one of which may occupy the ABO locus on each of the paired chromosomes. The O gene does not produce any demonstrable red cell antigen.¹ Antigenic determinants

of ABO blood groups are oligosaccharides located on glycoproteins and glycolipids expressed on erythrocytes and tissue cells and occur in various body fluids and secretions. Depending on an individual's ABO blood type, immunoglobulin M (IgM) antibodies directed against the missing A and/or B antigens are regularly present in serum; that constitute an immunologic barrier against incompatible blood transfusion and organ transplantation. The ABO gene codes for the glycosyltransferases that transfer specific sugar residues to H substance, resulting in the formation of

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blood group A and B antigens.² Transfusion of ABO-incompatible blood potentiates a greater risk for the recipients. ABO incompatibility accounts 37% of all reported transfusion-associated fatalities in the United States. Published reports cite an incidence of ABO discrepancy due to inappropriately identified specimens ranging from 1 in 517 to 1 in 3,400 samples.^{3,4,5} The true incidence of transfusion errors has been estimated to be as high as 5 times the number of detected errors, so the risk of mistransfusion may be severely underestimated.⁶

ABO discrepancies occur when unexpected reactions occur in the forward and reverse grouping. These can be due to problems with the patient's serum (reverse grouping), problems with the patient's red cells (forward grouping), or problems with both the serum and cells. The unexpected reaction can be due to an extra positive reaction or a weak or missing reaction in the forward and reverse grouping. Technical errors can also cause ABO discrepancies.⁷ Common Sources of Technical Errors Resulting in ABO Discrepancies are as follows: 1. Incorrect or inadequate identification of blood specimens, test tubes, or slides 2. Cell suspension either too heavy or too light 3. Clerical errors or incorrect recording of results 4. A mix-up in samples or Contaminated reagents 5. Missed observation of hemolysis 6. Failure to add reagents or sample 7. Failure to follow manufacturer's instructions 8. Uncalibrated centrifuge 9. Over or under centrifugation, Warming during centrifugation. If the initial test was performed using RBCs suspended in serum or plasma, repeat testing of the same sample using a saline suspension of RBCs can usually resolve the ABO discrepancy.⁸ Categories of ABO Discrepancies: ABO discrepancies may be arbitrarily divided into four major categories: group I, group II, group III, and group IV discrepancies.⁹

Group I Discrepancies: Group I discrepancies are associated with unexpected reactions in the reverse grouping due to weakly reacting or missing antibodies. These discrepancies are more common than those in the other groups listed. Common populations with discrepancies in this group are newborns, elderly patients, patients with leukemia, patients using immunosuppressive drugs that yield hypogammaglobulinemia or immunodeficiency diseases and patients with bone marrow or stem cell transplantations.

Group II discrepancies are associated with unexpected reactions in the forward grouping due to weakly reacting or missing antigens. This group of discrepancies is probably the least frequently encountered. Some of the causes of discrepancies in

this group include subgroups of A (or B), leukemia's may yield weakened A or B antigens, and Hodgkin's disease, weak reactions with anti-B antisera and is most often associated with diseases of the digestive tract (e.g., cancer of the colon). Rare Group II discrepancies: Weakly reactive or missing reactions in RBC grouping may be due to excess amounts of blood group-specific soluble (BGSS) substances present in the plasma, which sometimes occurs with certain diseases, such as carcinoma of the stomach and pancreas.

Group III discrepancies between forward and reverse groupings are caused by protein or plasma abnormalities and result in rouleaux formation or pseudo agglutination. Examples are elevated levels of globulin from certain disease states, such as multiple myeloma, Waldenström's macroglobulinemia or other plasma cell dyscrasias, elevated levels of fibrinogen, raised ESR, Wharton's jelly in cord blood sample and plasma expanders, such as dextran and poly vinyl pyrrolidone

Group IV discrepancies between forward and reverse groupings are due to miscellaneous problems and have the following causes: Cold reactive autoantibodies in which RBCs are so heavily coated with antibody that spontaneously agglutinate, independent of the specificity of the reagent antibody. Patient has circulating RBCs of more than one ABO group due to RBC transfusion or marrow/stem cell transplant. Unexpected ABO iso-agglutinins and non-ABO alloantibodies are also denoted in group IV discrepancies.

The delivery of this vital product 'blood' involves many people at different levels and different areas of the hospital. Errors can occur at any point. The first step in preventing mismatch is obtaining blood for pre-transfusion testing from the right patient and ensuring that all labeling is correct. Errors in these critical steps are recognized as the primary source of mismatching. It is imperative to recognize discrepant results and resolve them. Correct blood typing and labeling of an individual are essential to prevent ABO incompatibility. Different methods are available for determining ABO types of blood donors and recipients. Despite all the modern technologies and reagents availability, blood group discrepancies still occur. This study investigated the incidences ABO blood group discrepancy between red cells (forward blood grouping) and serum (reverse blood grouping) grouping of blood samples and tried to evaluate the risk factors. Therefore, the observations of this study will enrich our experiences in future as there are very few documents or data currently available to notify ABO discrepancies of our

country. That is why this study will help us to avoid single serious mismatched transfusion.

Methods:

This is a Cross sectional observational study done at Department of Transfusion Medicine of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. This study was conducted from November 2015 to April 2016 where two hundred recipient’s blood samples

were observed. All participants of this study were between the ages of 8 to 60 years except known cases of auto immune hemolytic anemia. The preliminary screening panel for each sample was included the age, sex, pregnancy, medication, lab tests and previous blood transfusion history. For identification of ABO blood group discrepancy, the following flow chart was executed:

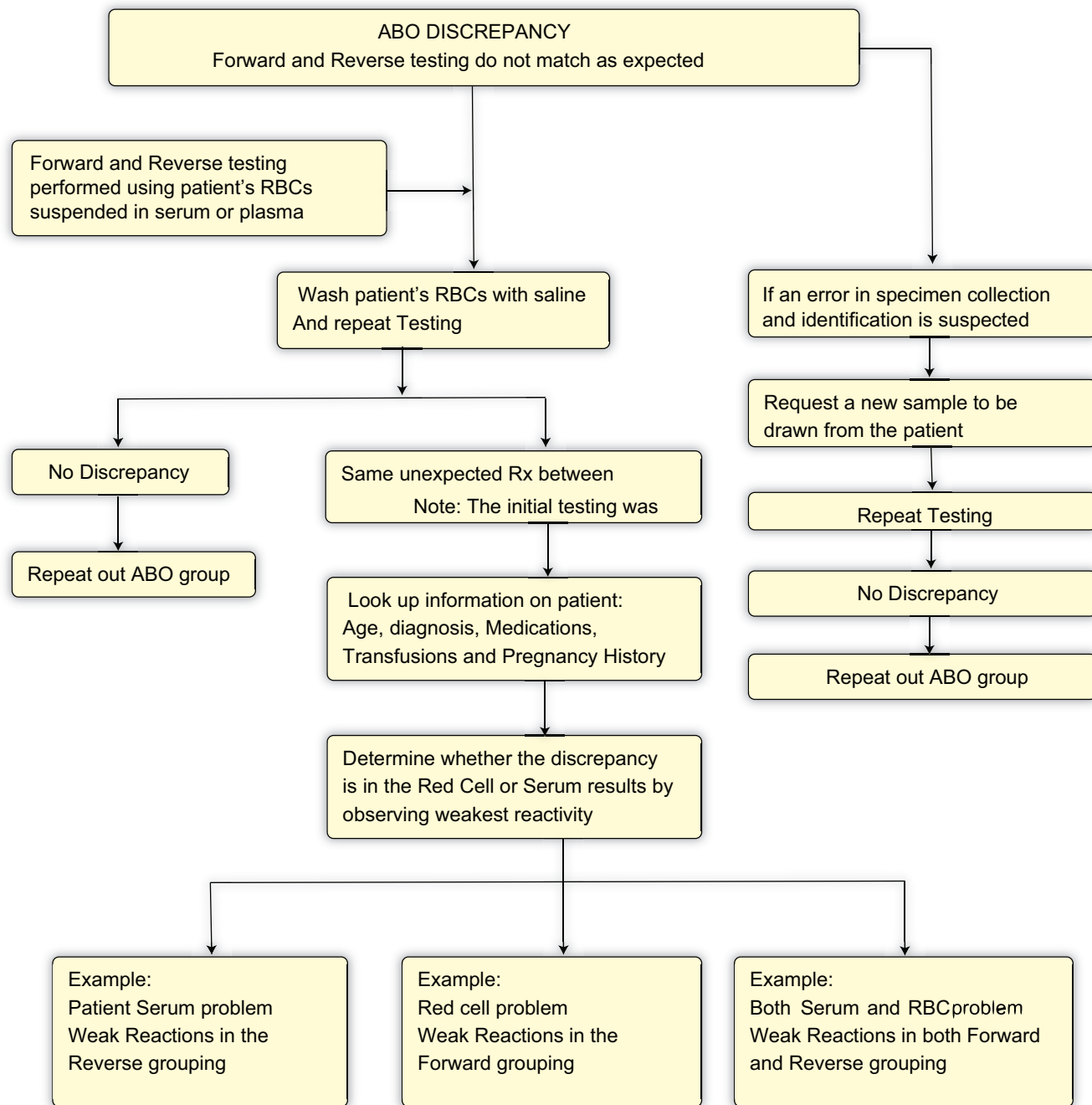


Fig.-1: Algorithm for identifying ABO discrepancies⁸.

Blood sample was collected in two pilot tubes from each recipient for cell and serum grouping. Seven to eight ml of EDTA blood was used for forward grouping and adsorption and elution techniques. Serum grouping was performed on clotted sample. ABO blood grouping was carried out by standard tube technique after washing the donor and reagent RBC's thrice with 0.9% normal saline. Cell grouping was performed using commercially available monoclonal anti-A and anti-B, anti-AB, antisera from two manufacturers (Tulip diagnostics and Span diagnostics) as per standard operating procedures and monoclonal anti-A, B sera (Tulip diagnostics) was used to confirm the routine findings. Serum grouping was performed using in-house pooled A cells, B cells, and screening O cells. Results of cell grouping, and serum grouping were matched. If there was any discrepancy, test was repeated with the same sample to rule out the possibility of technical errors. If it remained the same, then the possibility of a problem related to the sample was considered. Sample-related problem was divided further into two groups: ABO discrepancies that affected the ABO red-cell testing and those that affected the ABO serum testing. Those ABO discrepancies that affect either red-cell or serum testing was classified into whether an extra reaction was present or expected reaction was missing. Additional information pertaining to age, sex, pregnancy, history of previous blood transfusion, and medication was also obtained. Test was repeated on the fresh sample also. Supplementary reagents such as anti-A₁ (Tulip diagnostics) and anti-H (Tulip diagnostics) were also used. We also performed extended incubation at 4°C along with auto control and 'O' cells as a part of routine detailed serological workup for all samples where cell and serum grouping showed discrepant results. Polyclonal antisera of human origin from group B, group A, and group O individuals were used for adsorption to determine these subgroups. Heat elution technique using 6% bovine serum albumin was carried out at 56°C for 10 min and elute was tested against three un pooled reagent cells (A, B and O). In some tubes, agglutination was present after immediate spin, whereas tubes showing

no agglutination in the above step were incubated at 37 °C for 60 min. In these tubes, agglutination was observed after adding anti-human globulin reagent.⁹

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The quantitative observations were indicated by frequencies and percentages.

Results:

Table I
Distribution of the study patients (blood recipient) by age groups (n=200).

Age (years)	Number of patients	Percentage (%)
≤10	8	4.0
11-20	34	17.0
21-30	54	27.0
31-40	34	17.0
41-50	32	16.0
51-60	26	13.0
>60	12	6.0

Table I shows age distribution of the study patients (blood recipient). It was observed that majority 54 (27.0 %) of patients (blood recipient) belonged to age 21-30 years.

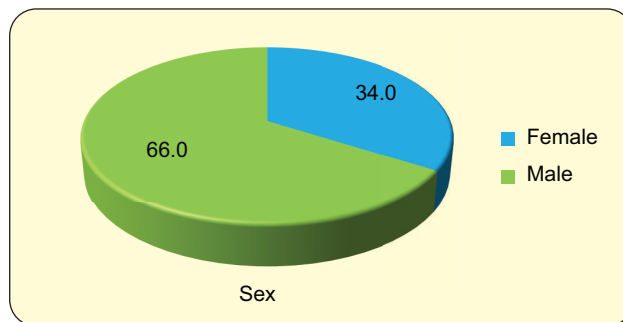


Figure 2: Pie chart shows sex distribution of the study patients.

Table II
Distribution of the study patients by ABO blood groups (n=200).

ABO blood groups		A	B	O	AB	A ₂ B
Forward blood group	Number of patients	40	80	55	24	1
	Percentage (%)	20.0	40.0	27.5	12.0	0.5
Reverse blood group	Number of patients	40	80	55	24	1
	Percentage (%)	20.0	40.0	27.5	12.0	0.5

Table II shows ABO blood groups of the study patients (blood recipient). It was observed that forward blood group was found to have 40(20.0%) patients in blood group A, 80(40.0%) in B, 55(27.5%) in O, 24(12.0%) in AB and 1(0.5%) in blood group A₂B. Reverse blood group was found to have 40(20.0%) patients in blood group A, 80(40.0%) in B, 55(27.5%) in O, 24(12.0%) in AB and 1(0.5%) in blood group A₂B.

Table III
Distribution of blood recipients by the presence of ABO discrepancies (n=200).

Presence ABO discrepancies	Number of patients	Percentage (%)
Present	11	5.5
Absent	189	94.5

Table III shows presence ABO discrepancies of the study patients (blood recipient). It was observed that presence ABO discrepancies were 11(5.5%) patients (blood recipient).

Table IV
Conditions with ABO discrepancies (n=200)

Causes of ABO discrepancies	Number of patients (blood recipient)	Percentage (%)
Transfusion Dependent	2	1.0
Thalassemia		
Mismatched transfusion	2	1.0
Autoimmune hemolytic anaemia	2	1.0
Multiple myeloma	2	1.0
Nonhematological malignancy (ovarian tumour)	1	0.5
ABO subgroup (A ₂ B)	1	0.5
Hematological malignancy (ALL)	1	0.5

Table IV shows risk factors of ABO discrepancies of the study patients (blood recipient). It was observed that 2(1.0%) patients (blood recipient) had TDT followed by 2(1.0%) had mismatched transfusion, 2(1.0%) had autoimmune hemolytic anaemia, 2(1.0%) had multiple myeloma, 1(0.5%) had non hematological malignancy ovarian tumour, 1(0.5%) had ABO subgroup (A₂B) and 1(0.5%) had ALL.

Discussion:

In this present study it was observed that majority 54 (27.0 %) of patients (blood recipient) were in 20-30 years age group. Similarly, Das et al.¹⁰ found a total of 14 patients aged between 18 and 64 years, which are

comparable with this current study. This current study observed that 132(66.0%) patients (blood recipient) were male and 68 (34.0%) were female. Male female ratio was 1.9:1. Similarly, male predominant also found by Giri et al.¹¹ where the authors observed 11554 subjects, among them 95.75% were male and 4.25% were female subjects which is opposite to the findings of Das et al.¹⁰ where male to female ratio was 1:2.5.

In this series of observation forward blood group was found to have 20.0% patients in blood group A, 40.0% in B, 27.5% in O and 12.0% in AB. On the other hand, reverse blood group was found to have 20.0% patients in blood group A, 40.0% in B, 27.5% in O, 12.0% in AB and 0.5% in blood group A₂B. Likewise, Maatoghi et al.¹² reported that the frequency of A and O phenotypes in white populations is 45.0% and 40.0%, respectively.

The distribution of ABO blood group varies regionally, ethnically and from one population to another. In the study of Giri et al.¹¹, the ABO blood group typing in the total sample showed the same trend of prevalence as in the general Indian subcontinent (B ≥ O > A > AB). In ABO system, their study showed the highest frequency of blood group B 31.89%, followed by O (30.99%), A (28.38%) and AB (8.72%). The investigators compared their result with other studies carried out in different countries of the world like Britain, USA, Nepal, Nigeria, Pakistan, Guinea, Saudi Arabia etc. Frequency of O blood group is highest in Britain 47.0%, USA 46.0%, Nigeria 54.2%, Guinea 48.9% and Saudi Arabia 52.0% and there is no marked difference in incidence of O blood group in these countries. In different part of India Warghat et al.¹³ and Rai et al.¹⁴ revealed that the frequency of blood group B (33.06%), followed by O (31.04%), A (27.02%) and AB (8.33%); and blood group B (42.0%), followed by O (30.04%), A (23.5%) and AB (4.0%) respectively.

In this study it was observed that presence of ABO discrepancies was 5.5% (11 blood recipients out of 200 samples). Besides this, it was observed that 1.0% patients (2 recipients) had TDT, 1.0% had mismatched transfusion, 1.0% had AIHA, 1.0% had multiple myeloma, 0.5% (1 recipient) had non-hematological malignancy (ovarian tumor), 0.5% had ABO subgroup (A₂B) and 0.5% had hematological malignancy (ALL). Similarly, Zhang et al.¹⁵ obtained, three conditions were related with the ABO blood type discrepancy, which included weaken antigen (2 cases), weakened antibody (3 cases) and ABO subtype (1 case). The satisfactory effect of transfusion was achieved in all patients with the principle of the same blood type or the compatible cross match.¹⁶

Decision to transfuse in autoimmune hemolytic anemia (AIHA) should be based on the pre-clinical condition of the patient rather than correcting the laboratory values. Moreover, delay in blood transfusion due to incompatible crossmatch, lack of adequate blood bank infrastructure, series of immune-hematological tests, lack of skilled person and critical patient condition make the transfusion management more challenging. More delay has been observed in patients with blood group discrepancy or patients suspected to carry underlying alloantibody due to previous blood transfusion or pregnancy.¹⁷ Blood group discrepancy in one patient was resolved using eluted red cells and adsorbed serum. Therefore, Das et al.¹⁰ concluded that decision to transfuse in AIHA should be based on the clinical condition of the patient. No critical patient should be denied blood transfusion due to serological incompatibility. All transfusion services should be capable of performing the minimum test required to issue “best match” PRBCs in AIHA. Specialized techniques such as elution and adsorption which are very much helpful in enhancing blood safety in AIHA should be established in all blood banks.

Furthermore, Gude et al.¹⁸ reported that discrepancies in ABO blood grouping can be catastrophic and even fatal. There are a few reasons that may contribute to ABO discrepancies other than clerical errors. Medical disorders such as liver disease and multiple myeloma may also contribute to such discrepancies. In acute leukemia, the A antigen may be weakened. Sometimes the blood appears to contain a mixture of group A and group O cells. In other cases, the red cells react weakly with anti-A. In a patient with erythroleukemia, of group B, 60% of the cells were not agglutinated by anti-B and appeared to be group O, but were very weak B, when separated from the normal B cells they would absorb anti-B.¹⁹

Conclusion:

For minimizing the event of hemolytic transfusion reaction, it is crucial to perform proper blood grouping both forward and reverse groups. These discrepancies can be avoided through detailed analysis of blood group typing. It will be beneficial not only for the patient but also the donor and will ensure the safety and efficacy of blood transfusion.

Limitations:

The study population was selected from one hospital in Dhaka city, so that the results of the study may not reflect the exact picture of the country. The present study was conducted at a very short period of time. Small sample size was also a limitation of the present study. Therefore, further study may be undertaken with large sample size.

Data availability:

The datasets of this study were not publicly available due to the continuation of analyses but will be available from the corresponding author on reasonable request.

Conflicts of interest:

The authors stated that there is no conflict of interest in this study.

Funding:

This research did not receive any fund.

Ethical Consideration:

Ethical approval has been taken before starting the study from the Ethical Review Committee of Bangabandhu Sheikh Mujib Medical University, Ref No: BSMMU/2016/2799.

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