

RESEARCH ARTICLE

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A close aspect of recognizing and resolving blood group discrepancy carried in a tertiary hospital, Bangladesh

Sonia Shormin Miah, Ashadul Islam, Mahbuba Sharmin Linia

ABSTRACT

Aims: Blood grouping includes both forward and reverse typing and there is always an inverse reciprocal relationship between this. Any unusual reaction can confer discrepancy and may lead to grave incompatible transfusion reaction. Resolving discrepancy is a crucial part that should not be ignored to save patients' life.

Methods: It is a cross-sectional study carried out at a tertiary hospital, over a period of one year. Sample had been selected based on comprehensive history, medical records, physical findings, and laboratory reports. ABO and Rh typing was done by using monoclonal immunoglobulin M (IgM) reagents (Tulip, Lorne and Biorex Diagnostic Ltd.) and A, B, and pooled O cell. Therefore, anti-H and anti-A1 lectin, extended red cell phenotyping, Coombs analysis had also done in few suspected cases.

Results: A total 25,082 blood group testing was done, 318 samples were carried out for group confirmation and discrepancy found in 51 cases. Technical and clerical errors were found in 18 cases. The remaining 33 samples reported as true discrepancy which was detected by Coombs analysis (both direct and indirect),

antibody screening, determination of Rhesus genotype and phenotype, and minor antibody detection. The rate of discrepancy is about 0.20%. Meanwhile, missing antigen was found in 3 cases (5.88%), missing antibody in 1 (1.96%), additional reaction due to rouleaux's in 4 (7.84%), mixed field (mf) reaction due to mismatched blood transfusion in 6 (11.76%), alloantibody with mixed phenotype in 5 (9.80%), cold antibody in 9 (17.24%), and Bombay in 5 cases (9.80%).

Conclusion: Blood group discrepancy is not an uncommon phenomenon. All the critical issues should be sensibly explored, evaluated, and analyzed by the expert physicians to avoid the discrepancy. These common but curable issues can be easily carried out and solved. A careful surveillance can also play a significant role in monitoring the recuperate misidentification problem. Therefore, all the technical staffs who usually play these vital roles should be mannered and clarified in a successful motion, to avoid this type of discrepancies.

Keywords: ABO discrepancy, Incompatible transfusion, Subgroup

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INTRODUCTION

ABO blood grouping and Rh typing has been established in 1900 by the famous pioneer Karl Landsteiner. This simplest plan of investigation that's

the human serum and cells of different individuals closely interacts to confound the reactions of antigen and antibody. These antigens have inverse reciprocal relationship with the antibodies. Some reactions cannot conclude the detection whereas some has unique pathology to overcome this type of situation [1].

Although all individuals of the same blood group have the same kind of agglutinin in their red blood cells, the sensitivity of the cells to agglutination varies from individual to individual. Moreover, there is a wide variation in the amount of agglutinin in the serum of different individuals belonging the same blood group.

Blood group discrepancy is not an uncommon issue to be afraid of, rather being a steady part to recognize the safety of the patients. Occasionally it closely correlated to the patient's age, his brief clinical history, drug, vaccination, pregnancy, and transfusion. The study of family tree is also become a part of this. Even in few cases patients' blood group has being draped by some disease condition or due to continuously mismatched blood transfusion. Therefore, immediate confirmation may not be possible whereas wait for few days up to three months until the acquired cells or drugs has not been eliminated by the reticuloendothelial system.

This study has shown the ways of blood group discrepancies that are commonly handled in this regional center. However, some samples show mixed field agglutination, missing of subgroup or any rare group identification, rouleaux formation. These all are the part of misidentification of blood group typing. Such types of problems are usually seen in patients suffering from acute infection, in conditions where the albumin-globulin serum protein balance is disturbed, even in normal blood when a smear is beginning to dry or when too concentrated cell suspension is used. When rouleaux formation is too heavy, it is sometimes difficult even for an unexperienced staff to distinguish it from true agglutination, as piles from compact masses are very similar to the appearance of agglutination.

In some cases, family tree analysis also guides us in a proper way to reach in an appropriate goal. All misleading errors are tried to solve; though most centers do not facilitate enough resources. Here in this study its being a worthy part to minimize the patient's reaction by identifying their problems clearly. Therefore safe transfusion was going on. In a study of Odisha, it has been recorded as 0.22% and another study from Heo et al. from Korea who found this about 0.14% [2, 3].

Clerical error is also a part of discrepancy. Mishra et al. found in their study about 35.09% [2]. The technical staffs who had attended to carry the samples failed to recheck this. Another problem is sample error as the registration number is same but the correct sample has been missing to reach the correct laboratory. Another issue is some samples have to be sent from the periphery, therefore appropriate history is missing. Moreover, some patients send their samples immediately after taking transfusion from a periphery and do not want to wait longer to

confirm their discrepancies which should be carried out to minimize their transfusion hazards. Meanwhile some elderly patients missing reactions also not clearly notified at early stage, therefore discrepancy attains.

Discrepancy invariably plays a crucial role in incompatible transfusion. An immune response following first transfusion may not be incidental to cause any discrepancy. Sometimes when a patient with alloantibody faced repeated blood transfusion from a remote area without getting indirect Coombs test (ICT) crossmatched blood or the antigen negative blood that should be the patient owned for. Therefore, this complicated situation usually arises when the alloantibody titer picks or multiple alloantibody formation starts. These all sometimes cause an unfavorable crossmatch with many donors. However, it has also been shown that some donor induced antigens cause alloimmunizations which may persist up to many years after the last transfusion. For example, if any patient has nurtured by Kell antigen from a donor and got blood transfusion again from the same donor, as in our country relative donors are more common than the voluntary donor, a life-threatening transfusion reaction may occur in a patient's life. That is the part of subsequent stimulation which actually proves the immune bystander reaction.

Blood group discrepancy can be alienated into four major categories I, II, III, and IV. Among them, group I discrepancies are associated with unexpected reaction in reverse grouping due to weakly reacting or missing antibodies. Group II discrepancies are associated with an unexpected reaction in the forward grouping due to missing or weakly reacting antigens. Both III and IV discrepancies are addition of all reactions in both forward and reverse grouping due to altered plasma protein ratio, cold, or unexpected is agglutinins [1, 4, 5].

Discrepancy that we found in missing reaction in forward grouping usually predisposes to group I. This frequency was studied by Mishra et al. (3.92%) and (1.7%) by Makroo et al. who have reported the subgroups of A (29%). In the study of Sharma et al., subgroups of A accounted for 19.6% of discrepancies [2, 6, 7].

Missing antibody is also another part of discrepancy which has been seen in a study of Mishra et al. who found the most common type of error missing or weak antibody 13.51%. The other difficulties associated with additional reaction, cold antibody, mixed phenotype that comprise in groups III and IV. In a study of Mishra et al. detection of cold antibody is about 8.11% [2].

These four groups are determined by the presence or absence of discrepancies that we should follow or to categorize the samples in which type they are placed for. If this basic procedure is followed, each sample will be clearly identified and the whole manipulation technique will ensure a minimum hazard to reduce the risk of errors.

MATERIALS AND METHODS

It is a cross-sectional study which was carried out in the Department of Transfusion Medicine, Bangabandhu

Sheikh Mujib Medical University, Dhaka from July 2018 to June 2019. Total 25,082 blood samples of patients were comprised in this study. Hemolyzed and lipemic samples are also included in this study. But the regular samples which do not require group confirmation are not included here.

What is the procedure carried out for this blood group confirmation?

Patient’s sample was received in the laboratory for confirmation of blood grouping when it was accompanied by hospital authorized blood requisition form duly filled and signed by a physician along with 3 mL of patient’s blood in each plain test tube as well as EDTA tube. For further rechecking the blood samples were stored at 2–6°C to prevent deterioration of weak antibodies or to avoid contamination.

In forward grouping monoclonal IgM (Tulip, Biorex and Lorne Diagnostic Ltd.), anti-A, anti-B, anti-AB, anti-D (IgG+IgM) and for reverse grouping freshly prepared A, B, and pooled O cell were done. All the tests were carried out by tube technique. Therefore, O_h phenotype was further confirmed by using anti-H lectin and performing secretor status which also confers as academic interest. Anti-A₁ lectin was used for the confirmation of A₂ subgroup in an individual to concur the allotted discrepancy.

For resolving the discrepancy, we also assembled for Coombs evaluation both direct and indirect according to the patient’s previous history of drug, pregnancy, diagnosed disease, transfusion, vaccination, or recovery from any recent viral infection, antibody screening, and extended red cell phenotyping.

RESULTS

A total 25,082 blood group testing was done where 318 samples were reserved for further group reconfirmation and among them discrepancy was found in 51 cases. Technical and clerical errors were found in 18 cases. The remaining 33 samples were reported as true discrepancy that has been resolved in both macro- and microscopically. Therefore, the rate of discrepancy was about 0.20% that has been recorded.

Meanwhile, some discrepancies were observed during the grouping of blood. They were: missing antigen in 3 cases (5.88%), missing antibody in 1 (1.96%), additional reaction due to rouleaux’s in 4 (7.84%), mixed field (mf) reaction due to mismatched blood transfusion in 6 (11.76%), mixed and missing phenotype in 5 (9.80%), cold antibody for both IgG and C3d in 5 (9.80%), alloimmunization with autoimmunization in 4 (7.84%), and Bombay in 5 cases (9.80%) (Table 1).

DISCUSSION

Safe blood transfusion is a pivotal part to maintain

Table 1: Distribution of reasons for ABO discrepancy (n = 51)

Type of discrepancy	Causes of discrepancy	n (%)
Forward typing	Missing reaction, subgroup of A, B, and AB (A ₂ , B _w , A ₂ B).	3 (5.88)
Reverse typing	Missing antibody	1 (1.96)
Additional reaction in both forward and reverse grouping	Rouleaux’s formation	4 (7.84)
	Cold antibody	5 (9.80)
	Mixed field reaction observed in blood grouping	6 (11.76)
	Alloimmunization with mixed Rhesus phenotype (mf)	5 (9.80)
	Alloimmunization with auto immunization (mf)	4 (7.84)
Others	Bombay (O _h)	5 (9.80)

the safety of the mankind. Detection of ABO blood groups through the agglutination technique is the basis of pretransfusion safety. However, weak agglutination reactions, that are the weak expression of A or B antigens on the red cell surface, may lead to discrepancy in blood grouping. The risk of blood transfusion has always been hidden due to this type of uneven full reactions rather than the other problems.

A total 25,082 blood group testing was done in this study. Therefore, the rate of discrepancy is about 0.20%. In a study of Odisha, it has been recorded as 0.22% which also shows a near comparison to this study. In another study from Heo et al. [3] from Korea who found this about 0.14% [3]. Similar study was also conducted by Esmaili et al. [5]. Their study results were an incidence of about 0.08% and 0.05%, respectively. In this study, 318 samples were sent for further group reconfirmation and among them discrepancy was found in 51 cases. Technical and clerical errors were found in 18 cases. The remaining 33 samples were reported as true ABO discrepancy that has been resolved [2, 3] (Table 2).

In our study the most common type of discrepancy that is ailed for sample-related error which confounds as 64.70%, whereas in a study from Mishra et al. found it about 64.91% [2] (Table 3).

In this study, we found technical error of 35.29% (Table 4). In the study of Mishra et al. it was 35.09%. Among the technical errors most common was mislabeling of samples which is about 11.76% and in a study of Mishra et al. found it apparently 5.26%. Others were reported as patient with same name 3.92%, patient carrying two or more registration number 5.88%, wrong sample in the test tube 7.84% (Table 5). In a study of Esmaili et al. found mislabeling of sample about 14.6% which is also near to our study [2]. These types of error are most

Table 2: Comparison of overall frequency of discrepancy of different published study

Study	Total number blood sample has been tested	Overall discrepancy	Frequency
Current study	25,082	51	0.20
Mishra et al. [2]	25,559	57	0.22
Shahshahani et al. [8]	322,222	130	0.04
Esmaili et al. [5]	549,229	261	0.05
Qutub et al. [9]	93,800	82	0.08
Heo et al. [3]	--	55	0.14

Table 3: Comparison of sample related error which causes discrepancy

Type of discrepancy	Number	Frequency (%)
Present study	33	64.70
Mishra et al. [2]	37	64.91

Table 4: Distribution of type of discrepancy (n = 51)

Type of discrepancy	Number	Frequency (%)
Technical error	18	35.29
Sample-related error	33	64.70

Table 5: Frequency of misidentification of samples among total discrepancies (n = 51)

Causes of misidentification of samples	Current study (n = 51) (%)	Mishra et al. [2] (n = 57)
Mislabeled	6 (11.76)	3 (5.26)
Patient with same name	2 (3.92)	1 (1.75)
Patient having common registration number.	3 (5.88)	2 (3.5)
Wrong sample in the test tube	4 (7.84)	--

common though they can be solved by training the staffs. Moreover, if we can improve our data system for patient enrolling, it could be avoided as the other developing countries do so.

Though ABO antibodies can be classified in so many ways and we have perceived in this present study; missing antigen found in 3 (5.88%), missing antibody 1 (1.96%), cold antibody for both C3d and IgG 5 (9.80%), and Bombay 5 (9.80%). In a study of Mishra et al. they found the most common type of error missing or weak antibody

13.51%, cold antibody 8.11%, and Bombay 18.92%.

Another common type of discrepancy that we found as well is missing antigen that we found in three cases. Among them, one was subgroup A₂, one was A₂B Rh negative, and the other was depressed antigenic function due to ongoing treatment with immunosuppressive drugs. The frequency was 3.92%. Mishra et al. found it to be 1.7%, Makroo et al. have reported that subgroups of A (29%) which was the second most common cause in their study [2, 4]. In the study of Sharma et al., subgroups of A accounted for 19.6% of discrepancies [7]. Weak subgroups of A perpetually have an inverse reciprocal relationship between the amount of H antigen on the red blood cell (RBC) and the amount of A antigens formed, the less H antigen expressed on the RBC. In presence of A₂ gene, only some of the H antigen is converted to A antigens and the remaining H antigen is detectable on the red cell surface. Testing with a monoclonal blend of anti-A, anti-B, anti-A₁, anti-H lectin; testing A, B, and H substances in the saliva of secretors, adsorption elution test, family study, and DNA-based study are other tests used for determining the blood group. Subgroups of B antigen are less common than A subgroups. Another weak reaction found in one case found an weakened expression of B antigen probably B₃ or B_w due to use of immunosuppressive drugs and the frequency is 1.92%. But secretor study reveals as weak B. Though molecular study could not be done due to the resource limitation. Meanwhile three cases (2.3%) of B subgroups were detected in a study of Makroo et al. In a study of Sharma et al., B subgroups were reported to be 4% [7]. Other studies have reported that B subgroups were more common in India in comparison to some other populations.

In this study we found missing antibody found in one case, therefore the frequency is 1.96%. This usually occurs due to weak or low avidity (anti-B or anti-A, B) antibodies. Shahshahani et al. found it in 15 cases and the frequency was 11.5%. Mishra et al. [2] found it about 8.72% cases, Makroo et al. found it in 7% cases among the blood donors, and Sharma et al. study, weak antibody was 58.8% and it was the most common cause of ABO discrepancy [7, 8]. Resolving weak or missing antibodies, we need to incubate the patient's serum for 15 minutes at room temperature to enhance antibody reactions and testing serum at 4°C for 15 minutes. Autologous control should be added to enhance the reactivity of the antibodies.

In this present study rouleaux formation is about 7.84% and Mishra et al. found it about 2.70%. It usually resolved by cell washing properly, repetition of both forward and reverse grouping. Saline replacement technique that is adding one to two drops of normal saline prior washing the sample also used to distinguish true and false agglutination [1, 2, 5].

Another issue was solving the patients' sample with mixed field (mf) agglutination, as the presence of two cell population in a single individual. In this study we found this due to mismatched blood transfusion 6 (11.76%).

The best way to solve it by repeating the blood grouping using different antisera. We found mixed and missing phenotype in 5 (9.80%) cases.

The extra red cell reactivity autoagglutinins which if present in the serum of an individual cause agglutination of own red cells. These are agglutinins which if present in the serum of an individual will cause agglutination of his own red cells. This phenomenon usually observed in lower temperature than 37°C, thus there is an autoagglutinin in the unknown serum active at the temperature of the test, the red cells will be agglutinated before being tested. This is usually observed at temperatures lower than 37°C and found in 9 cases which is about 17.64%. Mishra et al. found it about 35.1% and Heo et al. found it in about 5.5% and Makroo et al. found cold-reacting autoantibody (57%) and it was the most common cause of ABO discrepancy. Cold autoantibodies react usually all cells including screening cells, A, B, and autologous cells. An auto control is usually used to differentiate between cold autoantibodies from cold alloantibodies. If the auto-control is positive, the reactions observed with all cells. Washing red cells at 37°C or 45°C may disperse the agglutination and the red cells can be grouped in that way. In this study we also found a part of group discrepancy and mixed Rhesus phenotype about 5 (9.80%) cases [2, 3, 5].

Meanwhile, in this study we found O_h in 5 (9.80%) cases. Individuals lacking of all ABH antigens; therefore, they possess natural antibodies to A, B, and H. In forward ABO typing, initially they are typed as group O. In reverse typing, anti-H present in O_h individuals react strongly with group O red cells, which are rich in H antigen. Because the anti-H in O_h phenotype is capable of activating complement and causing intravascular hemolysis and featured as acute hemolytic transfusion reactions.

CONCLUSION

Although of considerable academic interest, solving blood grouping discrepancy is still a limited practice though it has worthy clinical importance. Anomalous grouping results primarily to the occurrence of any atypical reaction should be encountered sooner or later and must be dealt with according to the resolving procedure as we discussed above. We have also required to remember that the discrepancy is also closely related clerical and sample errors. Therefore, all the discrepancies should be investigated, monitored, and informed properly by the adequate history.

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Sonia Shormin Miah – Conception of the work, Design of the work, Acquisition of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Mahbuba Sharmin Lina – Conception of the work, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Guarantor of Submission

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Conflict of Interest

Authors declare no conflict of interest.

Data Availability

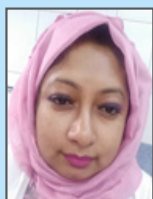
All relevant data are within the paper and its Supporting Information files.

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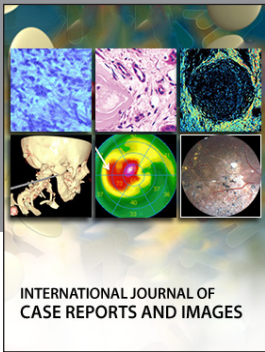
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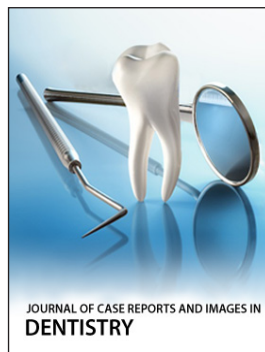
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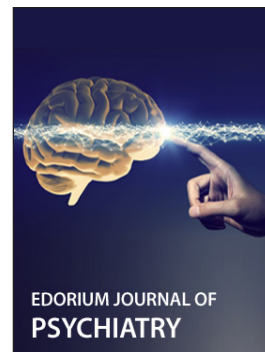
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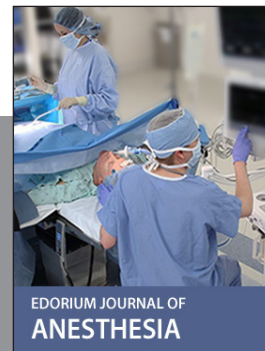
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