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A STUDY OF ABO BLOOD GROUPING DISCREPANCIES: EXPERIENCE OF A TERTIARY CARE HOSPITAL, NAVI MUMBAI

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ABSTRACT

Background: The ABO system plays a vital role in transfusion therapy and organ transplantation. ABO typing and compatibility tests are essential components of pre-transfusion procedures. ABO typing includes both cell and serum grouping, and discrepancies may arise in either category. Resolving these discrepancies is crucial for accurately determining the ABO and Rh groups of blood donors and patients. Failure to do so can result in ABO-incompatible transfusions, which may have fatal consequences. These discrepancies can arise from technical errors, protein abnormalities, or the presence of rare blood phenotypes, necessitating meticulous attention to detail and adherence to standard protocols. **Aim:** The aim of this study is to analyze the incidence and underlying causes of ABO typing discrepancies in blood transfusion services, with a focus on resolving these discrepancies to ensure transfusion safety. **Methodology:** A prospective study was conducted at MGM Medical College and Hospital, Navi Mumbai, analyzing 10,588 blood samples over a period of 12 months. We included both patient and donor samples, utilizing standard blood grouping techniques. Exclusion criteria were applied to ensure sample integrity. **Objectives:** To determine the incidence of ABO typing discrepancies in patient and donor samples. To identify causes of discrepancies: technical errors, protein abnormalities, rare blood phenotypes. To analyze the age and gender distributions of cases with ABO typing discrepancies. To determine whether standard protocols and communication are effective in resolving discrepancies. **Results:** The study revealed an overall discrepancy rate of 0.17%, with Type IV discrepancies being most prevalent (39%), followed by Type II (33%) and Type I (17%). Underlying causes included autoimmune hemolytic anemia, cold antibodies, and a rare blood group like the Bombay phenotype. We examined the age and gender distributions of cases, emphasizing the importance of effective communication and adherence to standard protocols in promptly resolving discrepancies to ensure transfusion safety.

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KEYWORDS: ***ABO typing discrepancies, Blood transfusion services, Transfusion safety, Technical errors, Protein abnormalities***

INTRODUCTION

ABO discrepancies arise when unexpected reactions occur during cell or serum grouping, potentially caused by issues with the patient's serum, RBCs, or both. [1] To prevent transfusion errors in these cases, quarantine the affected units and temporarily use group O red cells for patient transfusions until the issue resolves. [2] These discrepancies pose a serious threat to transfusion safety as the patient's blood group remains unknown, elevating the risk of acute hemolytic transfusion reactions, which are far more prevalent than transfusion-transmitted pathogens. [3, 4] Technical or clerical errors are often the primary cause of ABO discrepancies. [5]

These discrepancies are classified into four groups: Group I discrepancies involve unexpected reactions in serum grouping due to missing or weak antibodies, while Group II discrepancies involve similar issues in cell grouping caused by missing or weak antigens. Group III discrepancies occur due to protein or plasma abnormalities, leading to conditions like rouleaux formation and pseudo-agglutination. Group IV discrepancies encompass various miscellaneous causes, such as transfusion of out-of-group plasma, the presence of autoantibodies or cold alloantibodies, and pH-dependent or reagent-dependent antibodies. [6]

When encountering ABO discrepancies, it is crucial to repeat the test using the same sample to confirm the results. [7] In order to select compatible blood for transfusions, transfusion specialists and blood centre staff must conduct thorough investigations, run multiple tests, and verify blood group accuracy. [8] The documentation of any discrepancy outcomes is vital; however, the final interpretation should be put off until the discrepancies are addressed and resolved in order to ensure precise patient care and transfusion safety. [9]

MATERIALS AND METHODS:

A prospective study was conducted in the Department of Immunohematology and Blood Transfusion Medicine, MGM Medical College and Hospital, Navi Mumbai, Maharashtra, over 12 months from March 1, 2023, to February 29, 2024.

With the approval letter number DHR-EC/2023/03/05, the Institutional Ethics Committee accepted the study. The study included 5493 blood samples from patients requiring blood transfusions and 5095 blood donors. Of these, 3422 (67.16%) donated at blood donation drive camps, while 1673 (32.84%) made in-house donations. This study explored ABO discrepancies in samples obtained from patients and donors by performing forward and reverse blood grouping tests on samples obtained from inpatients, outpatients, and blood donors.

Inclusion criteria:

The blood samples, plain and EDTA vacutainer, from patients and donors were received in the Department of Immunohematology and Blood Transfusion at MGM Navi Mumbai for grouping and cross-matching.

Exclusion criteria:

Samples of newborns, infants up to 1 year of age, hemolyzed samples, clotted samples, and deferred donor blood samples.

The department of IHBT at MGM Medical College followed standard operational procedures (SOPs) to collect blood samples and perform blood grouping. The received blood samples will be checked for correct labeling, sufficient quantity for analysis, and hemolysis before being documented. Demographic data on the donors and the patients will be obtained from the requisition form submitted to the Department of Immunohematology and Blood Transfusion. Blood grouping was performed by conventional tube technique (CTT) and column agglutination method (BIO-RAD-Diaclone ABO/D+ Reverse Grouping). We used monoclonal antisera: anti-A, anti-A1, anti-B, anti-AB, and anti-H (Tulip diagnostic) for complete ABO grouping, along with in-house cells (group A, B, and O reagent red blood cells) for forward and reverse (serum) grouping, and anti-D-IgM monoclonal antibody (Rh typing) for Rh typing.

The methodology relies on the agglutination of red cells containing the specific antigen when exposed to corresponding antibodies (forward or cell grouping), as well as assessing the antibodies in serum that target the antigen in the pooled red cell preparation (reverse or serum grouping).

Cell grouping: We added one drop of the 2%–5% red cell suspension and one drop of the anti-A and anti-B reagents to each test tube.

Serum grouping: We added two drops of plasma and one drop of pooled A, B, and O cells to each tube.

Auto-control: The sample's red cells and serum reacted together.

The monoclonal anti-A1 (Dolichos biflorus) is used to distinguish the subtypes of the A-group.

Antibodies were screened using column agglutination technology (CAT) using a commercially available three-cell antigen panel (BIO-RAD) Coombs gel card. We used an extended eleven-cell (BIO-RAD) panel for antibody identification using low-ionic strength saline (LISS) if the antibody screening was positive. If a discrepancy has been identified, the clinical details of the patient or donor should be analyzed to ascertain the etiology, determine the type of discrepancy, and resolve the discrepancy.

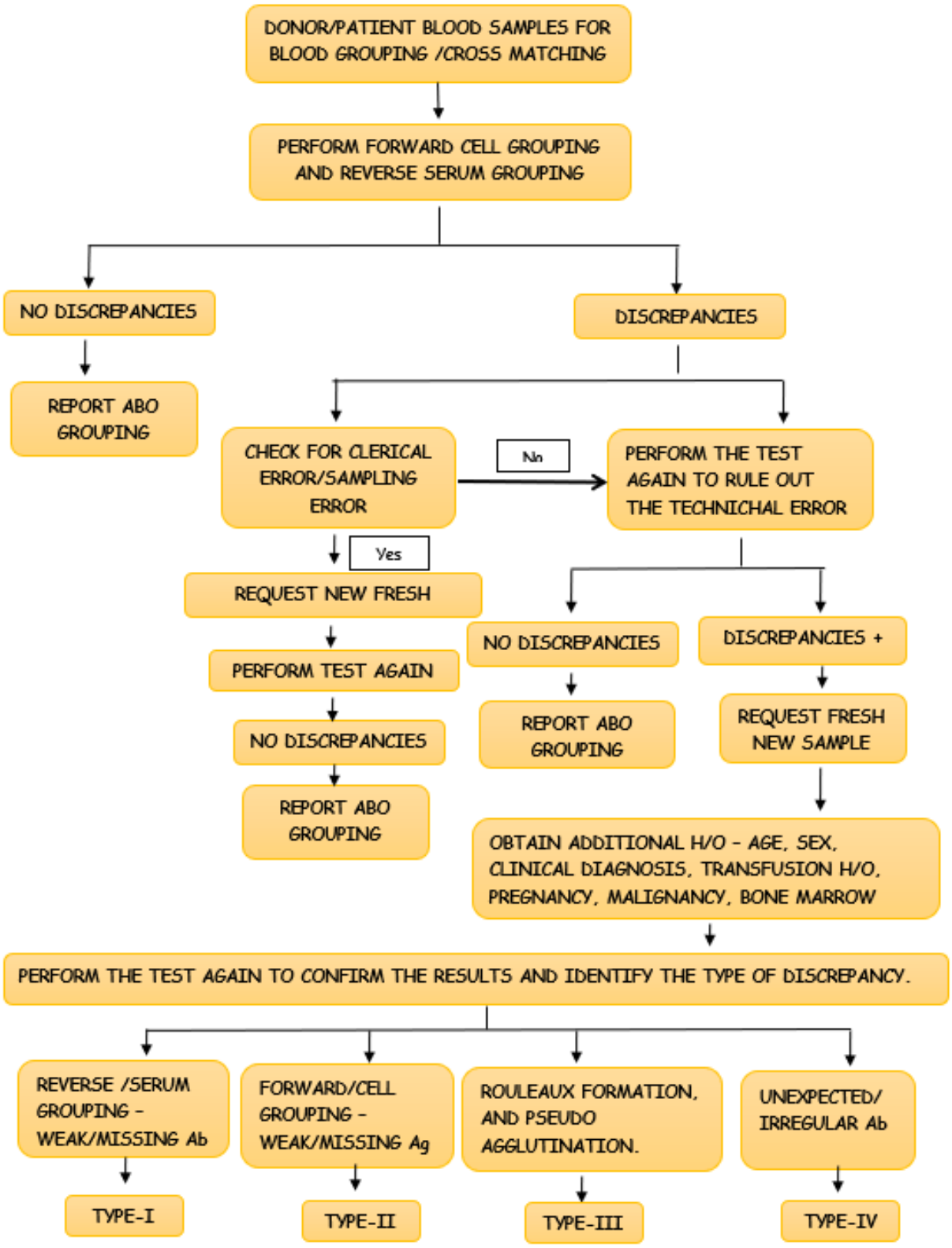

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Figure 1: Serological workup for resolution.

STATISTICAL ANALYSIS:

The collected data was subjected to a descriptive analysis, including various demographic characteristics of both the donors and patients. These details included their names, ages, genders, clinical diagnosis, transfusion and transplant histories, reasons for transfusion, medical histories, and medication histories. The statistical program in Microsoft Excel 2010 was used to determine the percentage and mean values. The discrepancies were classified into four distinct categories, namely Types I, II, III, and IV, for further analysis.

RESULT

A total of 10588 samples, 5493 from patients and 5095 from blood donors, underwent blood grouping testing during this prospective one-year study. ABO discrepancies totaled 18 (0.17%), with 13 (0.12%) found in patient samples and 5 (0.047%) in blood donors.

The study's donors consisted of participants ranging in age from 18 to 60, and the patients were more than 1 year old. The youngest was 17 years old, and the eldest was 73 years old (Table 1), with the overall mean age of ABO discrepancies being 38.3 years. In this study, out of 18 discrepancies, 9 (50.00%) cases were male, and 9 (50.00%) were female.

Out of 18 cases of discrepancies, 7 (39% of the total) were Type IV, with 6 (33%) diagnosed with AIHA and 1 (5.55%) in a healthy adult donor with the Bombay phenotype (Oh). Type II accounted for 6 (33%), including 1 (5.55%) with RTA undergoing E-Lap, 1 (5.55%) with DCLD having alcoholic cirrhosis, 1 (5.55%) with multiple myeloma undergoing bone marrow transplantation, 1 (5.55%) with breast carcinoma, 2 (11.1%) with subtypes of A, and 3 (16.7%) with a normal healthy donor with weak antibodies, 1 (5.55%) with community-acquired pneumonia, and 1 (5.55%) with pulmonary TB. The least common type is Type III, which occurs in 2 (11.1%) of normal healthy donors with rouleaux formation.

Table1: Age Distribution of patients and donors .

S.no	Age in years	No of patients and donors (n-18) had grouping discrepancies
1	>1-10 years	0(0%)
2	11-20 years	2(11.1%)
3	21-30 years	4(22.2%)
4	31-40 years	5(27.8%)
5	41-50 years	3(16.7%)
6	51-60 years	2(11.1%)
7	61 years & above	2(11.1%)

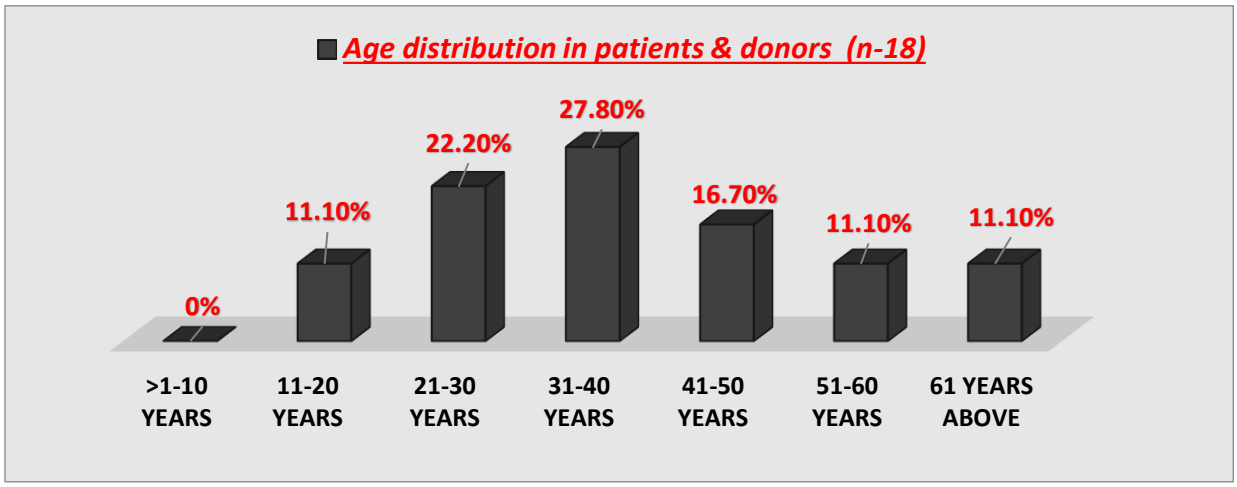

Figure 2 : Age Distribution of patients and donors .

Sex Distribution(n-18)

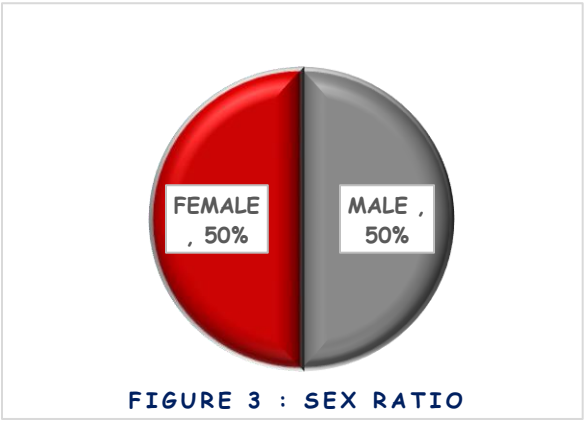

FIGURE 3 : SEX RATIO

Table 2 :Sex ratio.

Sex	Number of Patients & Donors (n18) %
Males	**9(50%)**
Females	**9(50%)**

Category Of ABO Discrepancies(n-18)

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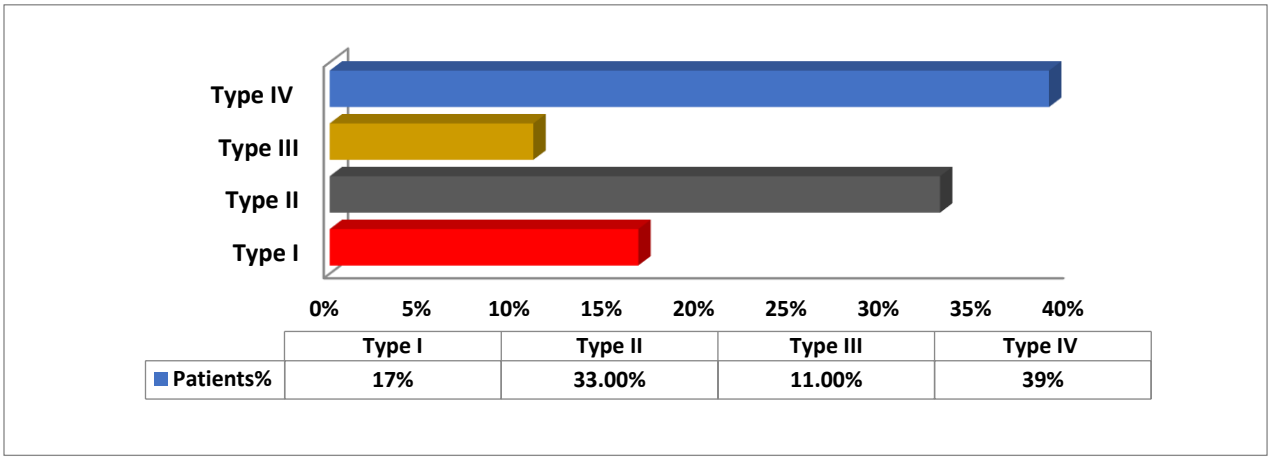

Figure 4 : Category of ABO discrepancies among patients and donors

CATEGORY OF DISCREPANCY	CAUSES FOR DISCREPANCY	DIAGNOSIS OF PATIENT & DONOR	RESOLVING BY	% PATIENT AND DONOR(n-18)
TYPE I	WEAKER ANTIBODY	COMMUNITY ACQUIRED PNEUMONIA	RESOLVED BY INCUBATING AT 37ºC FOR 1 HR	3(17%)
		PULMONARY TB	RESOLVED BY COLLECTING CLINICAL HISTORY AND DRUG HISTORY AND PERFORMING THE REPEATED GROUPING AND TYPING AND ICUBATING AT 4ºC FOR 30 min.	

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		NORMAL HEALTHY DONOR	**RESOLVED BY ADDING DOUBLE THE AMOUNT OF SERUM AND INCUBATING AT ROOM TEMPERATURE FOR 30 MIN.**	
TYPE II	**COLD ANTIBODY**	**RTA, E-LAP I/V/O HEMOPERITONEUM**	**RESOLVED BY PERFORMING THE REPEATED GROUPING AND TYPE AT 37ºC.**	**6(33%)**
	WEAKER ANTIGEN	**DCLD, ALCHOLOL CIRRHOSIS**	**RESOLVED BY INCUBATING AT 37ºC FOR 30 MIN**	
		MM, BMT	**RESOLVED BY INCUBATING AT 37ºC FOR 1 HR**	
		BREAST CARCINOMA ON CHEMOTHERAPY	**RESOLVED BY INCUBATING AT 37ºC FOR 1 HR & COLLECTING CLINICAL HISTORY AND DRUG HISTORY**	
	SUBTYPES A2B	**G2P1L1 CAME FOR BLOOD GROUPING**	**RESOLVED BY REACTING WITH ANTI-A1 LECTIN (AGGLUTINATION ABSENT)**	

	SUBTYPES A1	**NORMAL HEALTHY DONOR**	**RESOLVED BY REACTING WITH ANTI-A1 LECTIN (AGGLUTINATION PRESENT)**	
TYPE III	**ROULEAUX FORMATION**	**NORMAL HEALTHY DONOR**	**SALINE REPLACEMENT TECHNIQUE**	**2(11%)**
		NORMAL HEALTHY DONOR	**SALINE REPLACEMENT TECHNIQUE**	
TYPE IV	**MIXED ANTIBODY**	**AUTOIMMUNE HEMOLYTIC ANEMIA**	**RESOLVED BY INCUBATING AT 37ºC FOR 1 HR AND INCUBATING AT 4ºC FOR 1 HR**	**7(39%)**
	COLD ANTIBODY	**NSTEMI WITH AIHA**	**RESOLVED BY PERFORMING THE REPEATED GROUPING AND TYPE AT 37ºC.**	
		DIMORPHIC ANEMIA WITH AIHA	**RESOLVED BY PERFORMING THE REPEATED GROUPING AND TYPE AT 37ºC.**	
		CAD WITH HEMOLYTIC ANEMIA	**RESOLVED BY PERFORMING THE REPEATED GROUPING AND TYPING AT 37ºC.**	

	BOMBAY PHENOTYPE	**NORMAL HEALTHY DONOR**	**RESOLVED BY REACTING WITH ANTI-H (ABSENCE OF AGGLUTINATION)** **THE DONOR CALLED AGAIN FOR REPEATED BLOOD GROUPING TESTING, AND THE SECRETOR STATUS OF SALIVA SHOWS THE ABSENCE OF THE H ANTIGEN.**	

Table 3 : Category of ABO discrepancies among patients and donors .

RTA- road traffic accident , E-LAP – Emergency Laprotomy, DCLD- Decompensated Liver Disease, NSTEMI- Non ST segement Elevated Myocardial Infraction, CAD – Cold agglutination disease

Patients & donor details

S. NO	AGE	SEX	BG	DIAGNOSIS	CAUSES FOR DISCREPANCY	TYPE OF DISCREPANCY	RESOLVING BY	PATIENT /DONOR

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1.	**17**	**F**	**O +**	**AUTOIMMUNE HEMOLYTIC ANEMIA**	**MIXED ANTIBODY**	**TYPE IV**	**RESOLVED BY INCUBATING AT 37ºC FOR 1 HR AND INCUBATING AT 4ºC FOR 1 HR**	**PATIENT**
2.	**73**	**F**	**A +**	**COMMUNITY ACQUIRED PNEUMONIA**	**WEAKER ANTIBODY**	**TYPE I**	**RESOLVED BY INCUBATING AT 37ºC FOR 1 HR**	**PATIENT**
3	**63**	**M**	**B +**	**NSTEMI WITH AIHA**	**COLD ANTIBODY**	**TYPE IV**	**RESOLVED BY PERFORMING THE REPEATED GROUPING AND TYPE AT 37ºC.**	**PATIENT**
4	**26**	**M**	**B +**	**RTA,E-LAP I/V/O HEMOPERITONEUM**	**COLD ANTIBODY**	**TYPE II**	**RESOLVED BY PERFORMING THE REPEATED GROUPING AND TYPE AT 37ºC.**	**PATIENT**

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5	52	F	AB+	AUTOIMMUNE HEMOLYTIC ANEMIA	MIXED ANTIBODY	TYPE IV	RESOLVED BY INCUBATING AT 37ºC FOR 1 HR AND INCUBATING AT 4ºC FOR 1 HR	PATIENT
6	35	F	AB+	DIMORPHIC ANEMIA WITH AIHA	COLD ANTIBODY	TYPE IV	RESOLVED BY PERFORMING THE REPEATED GROUPING AND TYPE AT 37ºC.	PATIENT

7	42	F	B+	AIHA(MIXED)	WARM AND COLD AB	TYPE IV	RESOLVED BY INCUBATING AT 37ºC FOR 1 HR AND INCUBATING AT 4ºC FOR 1 HR	PATIENT
8	46	M	A+	DCLD, ALCHOLOL CIRRHOSIS	WEAKER ANTIGEN	TYPE II	RESOLVED BY INCUBATING AT 37ºC FOR 30 MIN	PATIENT
9	38	M	A+	CAD WITH HEMOLYTIC ANEMIA	COLD ANTIBODY	TYPE IV	RESOLVED BY PERFORMING THE REPEATED	PATIENT

							GROUPING AND TYPING AT 37ºC.	
10	**22**	**F**	**O+**	**MM, BMT**	**WEAKER ANTIGEN**	**TYPE II**	**RESOLVED BY INCUBATING AT 37ºC FOR 1 HR**	**PATIENT**
11	**23**	**F**	**B+**	**BREAST CARCINOMA ON CHEMOTHERAPY**	**WEAKER ANTIGEN**	**TYPE II**	**RESOLVED BY INCUBATING AT 37ºC FOR 1 HR MIN & COLLECTING CLINICAL HISTORY AND DRUG HISTORY**	**PATIENT**
12	**37**	**F**	**O+**	**PULMONARY TB**	**WEAKER ANTIBODY**	**TYPE I**	**RESOLVED BY COLLECTING CLINICAL HISTORY AND DRUG HISTORY AND PERFORMING THE REPEATED GROUPING AND TYPING AND ICUBATING AT 4ºC FOR 1 HR .**	**PATIENT**

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13	20	F	AB+	G2P1L1 CAME FOR BLOOD GROUPING	SUBTYPES A2B	TYPE II	RESOLVED BY REACTING WITH ANTI-A1 LECTIN (AGGLUTINATION ABSENT)	PATIENT
14	58	M	O+	NORMAL HEALTHY DONOR	WEAK ANTIBODY	TYPE I	RESOLVED BY ADDING DOUBLE THE AMOUNT OF SERUM AND INCUBATING AT ROOM TEMPERATURE FOR 30 MIN.	DONOR
15	32	M	O+	NORMAL HEALTHY DONOR	ROULEAUX FORMATION	TYPE III	SALINE REPLACEMENT TECHNIQUE	DONOR
16	42	M	Oh	NORMAL HEALTHY DONOR	BOMBAY PHENOTYPE	TYPE IV	RESOLVED BY REACTING WITH ANTI-H (ABSENCE OF AGGULTINATION) DONOR CALLED AGAIN FOR REPEATED BLOOD	DONOR

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							GROUPING TESTING, AND SECRETOR SATUS OF SALIVA SHOWS ABSENCE OF H ANTIGENTH	
17	**38**	**M**	**O+**	**NORMAL HEALTHY DONOR**	**ROULEAUX FORMATION**	**TYPE III**	**SALINE REPLACEMENT TECHNIQUE**	**DONOR**
18	**27**	**M**	**A+**	**NORMAL HEALTHY DONOR**	**SUBTYPES A1**	**TYPE II**	**RESOLVED BY REACTING WITH ANTI-A1 LECTIN (AGGLUTINATION PRESENT)**	**DONOR**

DISCUSSIONS

The ABO system is the most significant blood group system. ABO blood grouping is a fundamental diagnostic test that is vital for blood transfusion services. ABO grouping consists of both forward (cell grouping) and reverse (serum grouping) techniques, as well as the interpretation of the results that both methods must agree on.

ABO grouping plays a vital role in ensuring transfusion safety and reducing serious complications due to transfusions of incompatible blood. Patients should resolve ABO blood group discrepancies prior to transfusion of any blood or blood components, and donors should resolve the discrepancy before labeling their blood component with a blood type. Therefore, we conducted this study to analyze and assess the incidence and various causes of ABO typing discrepancies among patients and blood donors in our center.

ABO discrepancies can also occur due to clerical errors, such as patient sample identification, mislabeled samples collected for cross-matching techniques, and donor registration. When encountering a discrepancy, the first step is to identify the clerical error and repeat the test using the same sample. If the discrepancy persists, request and obtain a new, fresh sample before carrying out further tests to determine the cause of the discrepancies. Quality assurance of the reagents, meticulous technique, careful close observation, and proper interpretation of results can resolve many problems. We analyzed ABO discrepancies for both blood donors and patients who required cross-matching in our prospective study.

Type I discrepancy:

Out of 18 cases (n = 22), 17% (3/25) were type I discrepancies. Elderly donors and patients with immunocompromised or immunosuppressed conditions, such as community-acquired pneumonia and pulmonary TB, can exhibit absent or weakly reacting anti-A and anti-B antibodies. ABO antibodies are naturally occurring. Anti-A and anti-B antibodies are also formed after 4-6 months of age, followed by exposure to intestinal bacteria. Therefore, neonates and infants up to the age of 6 months typically do not undergo serum grouping. When performing grouping and cross-matching for neonatal transfusion conditions such as the double-volume exchange for congenital hyperbilirubinemia and hemolytic disease of the newborn (HDN) of Rh and ABO, or for infants requiring a top-up transfusion, it is always necessary to request a mother sample for cross-matching. Older individuals with decreased immunoglobulin levels often exhibit weak-reacting antibodies or no antibodies at all, leading to discrepancies in reverse grouping. Other conditions, such as gammaglobulinemia, congenital hypogammaglobulinemia, and chimerism, may also lead to weak or missing reactions that contribute to type I discrepancies.

Type II discrepancy:

Type II discrepancies were found in 33% (6/18) of cases (n = 22). Type II discrepancies are due to absent or weakly reacting antigens, which can be seen in subgroups of the A and B blood groups. Hematological malignancies, such as multiple myeloma and acute leukemia, as well as acquired B phenomena, can alter the antigens present on the surface of red cells. There are also reports of weakened ABO expression following pregnancy, post-transfusion of different blood groups, and post-allogenic bone marrow transplant with an ABO-incompatible donor. Molecular studies can demonstrate weak reactions or the absence of antigens. When comparing the A subgroup to the B subgroup, the A subgroup is the most common. The A blood group is primarily composed of two subgroups: A1 and A2. A1 is the most prevalent subgroup, accounting for up to 99% of the A type. Reacting with lectin (Dolichos bilflorus), which only agglutinates with A1 cells but not with A2 cells, can resolve the discrepancies caused by the A subgroup. One of the potential mechanisms that might explain the lack of or inadequate expression of A and B antigens is an increase in the expression of the H antigen. The inactivation of the A/B transferases prevents the conversion of the H antigen, the precursor of A and B antigens, into A and B antigens.

Type III discrepancy:

In our study, 11% (2/18) of cases lead to a type III discrepancy in the presence of the rouleaux formation. Patients with protein or plasma abnormalities commonly cause these abnormalities, leading to rouleaux formation, which the saline replacement technique resolves.

Type IV discrepancy:

Of the 39% (7/18) Type IV discrepancies, three were related to mixed antibodies, three to cold antibodies, and one to the Bombay (Oh) phenotype. Cold agglutinins resolve the discrepancies by repeating the test at 37 Â°C. It is important to inform the treating physician about the presence of a cold antibody in the patient. This reduces the risk of hemolytic reactions and transfusion-associated complications. Only when there is a clear indication and the patient is in a life-saving condition should we administer a transfusion; otherwise, we should withhold it. Individuals with the Bombay phenotype are considered rare because they do not express the H antigen on the RBC surface. The other conditions that contribute to this type are non-ABO alloantibodies, ABO isoagglutinin, and a recent history of IVIG infusion.

CONCLUSION

The first and foremost priority is to ensure the patient's safety by providing them with safe and compatible blood transfusions, as well as resolving ABO blood grouping discrepancies to prevent any fatal complications. When it comes to blood transfusion services, the ABO blood grouping is the initial and most important investigation. While encountering ABO discrepancies, the initial action to take is to rectify any clerical errors. This is followed by doing basic serological tests such as the direct Coomb's test, the indirect Coomb's test, and auto control. Serological methods may handle many of these issues, minimizing the need for more advanced investigations. It is crucial to closely follow the standard operating procedures, including sample collection and laboratory techniques, in order to prevent delays in turnaround time for issuing blood components. If there is a delay in resolving the discrepancy, the clinician and the transfusion medicine specialist must effectively communicate and coordinate to discuss the clinical history, laboratory values, diagnosis, ongoing treatment, and selecting the appropriate blood components for transfusion. Molecular ABO genotyping simplifies the identification of rare subgroups, such as the Bombay and Para-Bombay phenotypes. This is because it lets us improve our basic red cell serology techniques to deal with these unusual ABO discrepancies.

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