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### **Original Article**

# Prevelance of ABO Discrepancies and Its Classification in Blood Donors at Regional Blood Centre, Karachi

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### Abstract

**Background:** ABO and Rh systems are the most important blood group systems of human body. More than 400 blood group systems have been reported, however majority of them are not clinically significant. The ABO group system comprises of antigens represented by forward blood grouping and antibodies represented by reverse blood grouping. Reverse blood grouping is usually done to verify the results of forward blood grouping. ABO discrepancy is defined as a difference of result between forward and reverse group and has been reported to be around 0.15-0.19%. ABO discrepancies are divided into 4 types. Type 1 discrepancy include those having missing antibodies in reverse grouping and are most common. Type 2 discrepancy include missing antigens in forward grouping. Type 3 are those because of abnormalities in plasma or proteins and type 4 is represented as miscellaneous.

Aims and Objectives: The aim of this study was to calculate the frequency of ABO discrepancies in healthy blood donors, its classification into different types and identification of different subgroups in order to provide safe blood to the patients.

Material and Methods: Descriptive, cross sectional study was conducted at Regional blood centre Karachi from January 2020 to August 2023.78,623 donors were enrolled in the study. Blood samples were collected in di- potassium ethylene diamine tetra acetic acid (K2 EDTA) and yellow gel top vacutainers. Blood grouping was performed by a technician/ technologist by tube method and was reconfirmed by a senior technologist.

Results: 78623 samples were included in the study, with majority of the males. The mean age of blood donors was 28.5 years. ABO discrepancy was found in 108 cases (0.13%). 4 groups of ABO discrepancies were made according to AABB. Most common ABO discrepancy was type 4 (46.3%) followed by type 1 (37.0%). Type 2 and type 3 were 11.1% and 5.6% respectively. Most common blood group associated with ABO discrepancies was O positive, followed by A positive (p value 0.001). Most subgroups were associated with a followed by B.

Conclusion: ABO blood grouping remains the most important testing in the blood donors. The significance of determining the actual blood group lies in the fact that safe blood transfusion should be the utmost priority. Complete blood grouping along with resolution of discrepancies will continue to be a significant feature in providing safe blood to the patients and preventing them from fatal transfusion reactions.

### **INTRODUCTION**

ABO Blood Group is the most common, clinically significant and important group for safe blood transfusion in blood banking. ABO system is of 4 basic types (A, B, O and AB).

The basis of ABO grouping is of two antigens- Antigen A and Antigen B. The ABO grouping system is classified according to the presence or absence of antigens on the red blood cells surface and plasma antibodies.

- · Group A contains antigen A and antibody B.
- · Group B –contains antigen B and antibody A.

- · Group AB -contains both A and B antigen and no antibodies (neither A nor B).
- Group O contains neither A nor B antigen and both antibodies A and B [1].

In ABO blood grouping, forward and reverse groups are performed for the detection of ABO antigens on red cell and antibodies in serum respectively [2]. For detection of ABO Antigen on red cells, forward Grouping is performed with commercially prepared monoclonal anti-A and anti-B antisera's whereas for detection of anti-A and anti-B antibodies in the serum we perform reverse grouping using the laboratory prepared red cells in order to confirm the results of forward grouping [3].

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The ABO blood group system is significant because mismatching of the blood group can cause clumping of red cells/ reactions that can lead to significant hemolysis. As for group A, we can give a blood or O blood (due to absence of any antigen) but not any other blood group [4]. Similarly, in AB patient, any group can be given due to the presence of both A and B antigens, therefore, group O is called us universal donor and AB as universal recipient.

ABO Blood Group discrepancies are noted when the results of forward and reverse grouping differs from one another either due to an unexpected extra reaction or a missing reaction either in forward or reverse grouping [5]. Normally, red cell and serum typing gives strong reactions in donor and recipients, mostly (3+ to 4+) reactions. However, in ABO discrepancies weak reactions (1+ to 2+) are seen. Most important reason for these discrepancies can be technical errors by the technologists, but the true discrepancies occur due to intrinsic problems within red cell antigens or serum antibodies such as weak subgroups, autoantibody, alloantibody or abnormal proteins. It is very crucial to resolve these discrepancies through detailed history of the patient/ donor along with proper and detailed serological workup before establishing the actual blood group and starting the blood transfusion [6].

ABO Discrepancies have four major types:

Type I: Unexpected reactions in reverse grouping due to missing or weak antibodies, most common type of discrepancy.

Type II: Unexpected reactions in forward grouping due to missing or weak antigens.

Type III: these discrepancies occur due to plasma/protein abnormality and usually give pseudo agglutination in both forward and reverse grouping.

Type IV: are termed as miscellaneous due to different issues resulting in false positive or negative reactions in either forward or reverse grouping [7].

Resolution of these four discrepancies depends upon the type and include methods like changing serum to cells ratio, incubation at room temperature, 37 degrees Celsius and 4 degrees Celsius and elution and adsorption [8].

The data regarding ABO discrepancies is still not much reported due to paucity of reverse grouping in majority of blood banks in Pakistan. The significance of the resolution of discrepancy lies in the fact that a wrong blood group can causes severe hemolytic reaction and death of the patient [9] so reporting and resolution of ABO discrepancy is the first step in assuring safe blood transfusion.

### **MATERIALS AND METHODS**

Descriptive, retrospective study was conducted at Regional blood center Karachi (RBC-KHI) from January 2020 to August 2023. A total of 86,066 donations were received from the year 2020 to 2023. Out of total, 7443 were excluded due to the reason

of reactive viral history and/or missing data and 78,623 were included in the study. There were 78593 males and 30 females.

Blood samples were collected in di-potassium ethylene diamine tetra-acetic acid (K2 EDTA) and yellow top gel vacutainers. Blood grouping was performed by a technician/technologist and then reconfirmed by a senior technologist. For ABO typing the tube method was used. Monoclonal antisera: anti-A, anti-B and in-house cells (group A1, B, and O red blood cells) were used for forward and reverse grouping, respectively. ABO typing was performed according to standard operational procedures (SOPs) of the Regional Blood Center (RBC-KHI). In all discrepant cases, technical/clerical errors were investigated first. Repeat ABO typing was performed on the same sample and on a new sample using the standard tube method. After ruling out technical/ clerical errors, problems were studied with red blood cells or plasma. Frequency of distribution of blood group types and types of discrepancies were given and Chi-square test of association was applied to observe significant association of blood group types and types of discrepancies.

### **RESULTS**

Over all ABO discrepancy was observed in 108/78623 cases (0.13%). The frequency of discrepancy in 2021 to 2023 years was 2/18922 (0.01%), 74 /32199 (0.23%) and 29/26055 (0.11%) respectively and no discrepancy was found in the year 2020. Types of discrepancy overall and in the years are depicted in Figure 1. The most common type of frequency overall, was type-4 50(46.3%) followed by type-1; 40(37.0%), type-2; 12(11.1%) and type-3 6(5.6%) respectively. The ABO discrepancy data is presented in Table 1. The association of type of discrepancy and blood group was evaluated and it was found that the highest frequency of type-4 as well as type-1 discrepancy was observed in O-Positive and most common subgroups were of a group. Chi-square test of association was applied to observe significant association of blood group types and types of discrepancies; it was found statistically significant (p=0.001) as shown in Table 2. Statistical analysis was done on SPSS version 23.

### **DISCUSSION**

ABO discrepancies are reported to be somewhere around 0.05

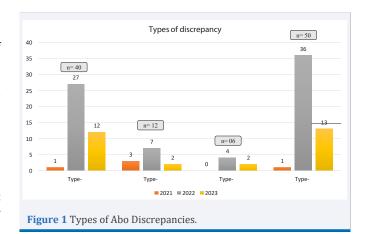


Table 1: Abo Discrepancy Data

Overall (n= 108)						
Age in years	Mean					
	2021	05	4.6			
Yearly distribution	2022	74	68.5			
	2023	29	26.9			
Type of discrepancy	Type 1	40	37.0			
	Type 2	12	11.1			
	Type 3	06	5.6			
	Type 4	50	46.3			
Discrepancy found	Donor	108	100			
in	Patient	Not included				
C 1	Male	105	97.2			
Gender	Female	03	2.8			
	A -	1	0.9			
	A +	28	25.9			
	A2 +	5	4.6			
	A2B +	2	1.9			
	AB +	7	6.5			
	Am subgroup	1	0.9			
Blood group	В -	1	0.9			
Interpretation	B +	19	17.6			
-	B Subgroup +	6	5.6			
	0 +	33	30.6			
	0 -	1	0.9			
	A Subgroup +	1	0.9			
	A subgroup +	2	1.9			
	A3 Sub group +	1	0.9			

Table 2: Association of Types of Discrepancies with Blood Groups

Blood Group Interpretation	Type 1	Type 2	Type 3	Type 4	P-value
A -	0	1	0	0	
A +	12	3	0	13	
A2 +	2	0	1	2	
A2B +	1	0	0	1	
AB +	1	0	0	6	
Am subgroup	0	1	0	0	
В -	0	0	0	1	
B +	3	1	3	12	0.001
B Subgroup +	4	2	0	0	
0+	15	1	2	15	
0 -	1	0	0	0	
A Subgroup +	1	0	0	0	
A subgroup +	0	2	0	0	
A3 Sub group +	0	1	0	0	

to 0.09%mainly due to lack of proper testing and identification of discrepancies [10]. There is paucity of data of ABO discrepancy specially in healthy blood donors. Our data represents it to be 0.13% and is more than the data published in different regions of our country and our neighboring countries. In this study, we excluded any technical/ clerical errors and focused on 4 types of true ABO discrepancies. One study was conducted by Hayedeh etal [11] at Regional blood centre of Iran, it reported the ABO discrepancy rate to be 0.04%. It was also conducted on healthy blood donors and they included the technical errors. Our study has more frequency of discrepancies than the study conducted in Iran, it could be due to various facts including ethnicity, history of donor, blood testing techniques and certain medicines/drugs

taken by donors. Sumera etal [12] conducted a study at Ziauddin hospital that showed ABO discrepancy to be 1.1% which is more than our study and few other studies. It could be due to the fact that they conducted study on the patients and not the healthy donors as well as their sample size was relatively small. Our study showed the highest frequency of Type 4 ABO discrepancies which is contrary to majority of other studies. Hayedeh etal didn't categorize their results as type of discrepancies, instead they represented the results according to the cause. One study was conducted by Ghazala etal [13] at Lahore and it showed Type 2 as the most common type of discrepancy, however, it was done on the patients and not donors. Other study was conducted by Sumera etal [12] at Ziauddin hospital Karachi on patients and they reported Type 1 discrepancy as most common. G. kaur [14] etal conducted another study on blood donors reporting the frequency to be 0.06%. They also included the technical errors and apart from that they reported subgroups of A and B to be most common, Hayedeh etal also reported subgroups of A to be most common. Our study was also in concordance with these data and we reported 9.2% of subgroup A. We had 6 (5.5%) cases of subgroups of B, Hayedeh et al and Sharma et al [15] represented subgroups of B to be 2.3 [11] and 4.4% which is almost similar to our study. We also identified the rare group of Bombay (Oh) in 4 healthy blood donors, who were previously not registered with any of the blood centres in Pakistan. Makroo etal [6] conducted a study on patients as well as donors and reported cold autoantibodies to be the commonest of ABO discrepancies. As cold autoantibodies are type IV discrepancy, it is in concordance with our study, as we also found retrospectively that majority were cold autoantibodies specially associated with group O. The next common type of discrepancy was reported to by Type 1 due to missing reactions in reverse grouping.

Worldwide, type 1 discrepancies are most common and are mainly due to extreme of ages. Sumera etal [12] also concluded type 1 discrepancy to be most common in patients. As our study was mainly focused on blood donors, extremes of ages, hyopgamaglobeneimia was not a major concern. For all of these discrepancies, we tried to resolve as per our SOP's, which are in accordance with AABB. We used anti A1 lectin, Anti H, Anti A, B as well as pre warm, elution and adsorption techniques when necessary.

Overall, the ABO discrepancies are not well identified in many centres of our country due to lack of proper testing that can lead to serious hazards in patients. Moreover, in Pakistani blood transfusion units, a blood is not allowed to release until the discrepancy is resolved, however there is no consolidated plan to inform these donors about their actual blood group, also many of the blood centres are not performing reverse grouping till date which is further dangerous for patients as well as donors (as they are labelled wrong blood group). It is therefore, essential to resolve and label them accordingly [16].

### **CONCLUSION**

In order to decrease the chance of hemolytic transfusion reaction, it is essential to perform proper blood grouping both

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forward and reverse groups, and if any discrepancy occurs between two, it has to be completely resolved. It will be beneficial for both patient as well as the donor and will increase the safety and efficacy of blood products.

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