DOI: 10.7860/JCDR/2024/67742.19015

Original Article

Transfusion Medicine Section

Estimation of ABO Anti-A and Anti-B Agglutinin Titers among Blood Donors at a Tertiary Care Referral Teaching Hospital Blood Centre in Southern India: A Cross-sectional Study

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ABSTRACT

Introduction: Transfusion of blood Group O and its components with high ABO antibody titers to non O recipients has been shown to cause acute transfusion reactions, especially in platelet transfusions, as platelets contain significant amounts of ABO antigen on their surface as well as Anti-ABO alloisogglutinins in plasma.

Aim: To estimate the ABO Anti-A and Anti-B agglutinin titers among blood donors at a tertiary care referral teaching hospital blood centre.

Materials and Methods: This cross-sectional observational study was conducted at the Immunohaematology laboratory of the Department of Transfusion Medicine, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India, from March 2021 to June 2022. All blood donors presenting to the blood centre were screened for eligibility for blood donation. Donors who fulfilled the eligibility criteria as per the Drugs and Cosmetics Act, 1940, and Rules, 1945 were included. Anti-A and Anti-B titers were determined by the conventional tube method with dilutions of 1 in 2, 1 in 4, 1 in 8, 1 in 16, 1 in 32, 1 in 64, 1 in 128, 1 in 256, 1 in 512, and 1 in 1024. A titer of <64 was considered as a low titer, while a titer of >64 was considered as a high titer.

Results: A total of 399 donors were included in the study, with 393 (98.5%) being males and 6 (1.5%) being females. The mean age of the study population was 28.49 years. Among blood group A, the percentage of individuals with an IgM anti-B titer of <64 was 85.44% (88) and >64 was 14.56% (15), whereas in blood group B, IgM anti-A titers of <64 were 65.5% (93) and >64 were 34.5% (49). In blood group O, the percentage of individuals with <64 and >64 titers of IgM anti-A were 52.60% (81) and 47.40% (73), respectively, whereas for anti-B, <64 and >64 titers were 77.92% (120) and 22.08% (34), respectively.

Conclusion: It is recommended that a database be maintained in all institutes by estimating antibody titers for every A, B, O blood group donors. Whole blood or platelets from group O donors with IgM Anti-A and Anti-B antibody titers <64 can only be transfused across the ABO barrier. As the majority of group A donors had titers <64, group A platelets may be transfused across the ABO barrier in emergency situations.

Keywords: Blood groups, Haemolysis, Platelet transfusion, Transfusion reactions

INTRODUCTION

Since the discovery of the ABO blood group by Karl Landsteiner in 1900 [1], transfusion medicine has continued to evolve, with more than 600 red cell antigens and different classes of antibodies are identified so far [2]. The use of blood Group O transfusions to patients of all groups has continued since the Second World War; nevertheless, the transfusion of Group O plasma to Group A recipients sometimes causes severe red cell destruction [3]. Transfusion of blood Group O and its components with high ABO antibody titer to non O recipients has been shown to cause acute transfusion reactions and other unwanted transfusion-related outcomes like haemoglobinemia, jaundice, progressive anaemia, spontaneous agglutination, positive direct antiglobulin test, and increased osmotic fragility of the patient's red cells [3-5]. Particularly, when it comes to platelet transfusion therapy, problems may arise because the platelet components contain both significant amounts of ABO antigen on their surface, as well as anti-ABO alloisogglutinins in the donor's plasma [3]. Moreover, the majority of blood centres internationally do not include a titration method to limit the risk of haemolysis when platelets containing ABO-incompatible plasma must be transfused [6,7]. On the other hand, various studies report that a potential risk does exist when ABO-incompatible platelet units, containing "high-titer" anti-A and anti-B antibodies, are transfused. The risk is even greater when Group O platelet components are transfused out of the group [8-11]. Some studies have also suggested the role of the environment and diet in influencing the levels of these antibodies [12]. As variables specific to donors and environmental factors are associated with changing anti-A and anti-B titer levels in donors, a regular survey on ABO antibody levels in the donor population should be performed for each country and ethnic group [13,14]. This is coupled with an insufficient amount of data on titer levels in the Indian population, with only a few reports about them [15-17]. The objectives of present study were to estimate the ABO anti-A and anti-B agglutinin titers among blood donors to determine the baseline values and to create a database of blood donors having low titers of ABO antibodies.

MATERIALS AND METHODS

This cross-sectional observational study was carried out at the Immunohaematology laboratory of the Department of Transfusion Medicine, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India, from March 2021 to June 2022. All blood donors presented to the blood centre were screened for eligibility for blood donation. Samples were collected every Monday and processed in the same week. Institutional Ethics Committee (IEC) approval was obtained before starting the study (Roc. No. AS/11/IEC/SVIMS/2017), dated March 20, 2021.

Inclusion criteria: Donors who fulfilled the eligibility criteria as per the Drugs and Cosmetics Act, 1940 and Rules, 1945 [18] were included in the study after getting the written informed consent.

Exclusion criteria: AB blood group donors, donors with irregular red cell antibodies, those reactive for HIV, Hepatitis-B, Hepatitis-C, Malaria, and Syphilis, and those who had not given consent were excluded from the study.

Sample size calculation: The sample size was calculated using a clinically relevant percentage of 46%, which was derived from previous studies [19,20]. The sample size was calculated using the appropriate formula [21] and resulted in a total of 382 participants.

Study Procedure

Blood grouping, antibody screening, and transfusion-transmitted infection screening were conducted for study participants. Antibody titer for ABO anti-A and anti-B antibodies were determined for all eligible study population by conventional tube method. The serum was separated, and dilutions were done by saline double dilution technique. The dilutions were immediately processed at room temperature by centrifugation at 1000 rotations per minute for one minute. Polled A1 cells and B cells were prepared using in-house collected blood units. Testing was carried out for dilutions at 1 in 2, 1 in 4, 1 in 8, 1 in 16, 1 in 32, 1 in 64, 1 in 128, 1 in 256, 1 in 512, and 1 in 1024. The titer was interpreted as the reciprocal of the highest dilution that produced a 1+ macroscopic reaction in the tube. A titer of <64 was considered a low titer, while a titer of >64 was considered a high titer as per the published literature [18,19].

STATISTICAL ANALYSIS

Data was entered into a predesigned proforma and later into Microsoft Office Excel (Microsoft Corporation, Redmond, WA). All continuous data was expressed as mean, standard deviation, and median with interquartile range as appropriate. Continuous data was analysed using the Student's t-test/Mann-Whitney U test as appropriate. Categorical data was expressed as percentages and was analysed using Chi-square/Fisher's exact test. A p-value of <0.05 was considered statistically significant. The data was analysed with Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS, Inc., Chicago, IL).

RESULTS

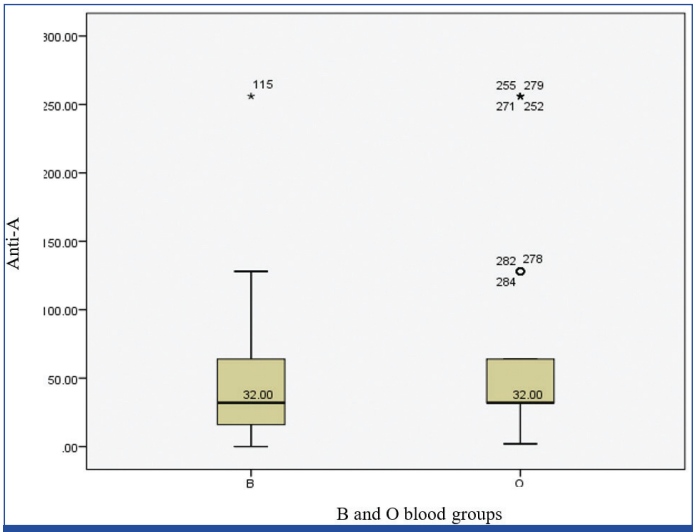
During the study period, a total of 487 blood donors were enrolled in the study, of whom 399 fulfilled the inclusion criteria. The remaining 88 were excluded from the study based on the exclusion criteria. Among the study population, 393 (98.5%) were males, and 6 (1.5%) were females.

The age range was 18-60 years (mean age: 28 years), with the majority of patients were in the 18-30 age group (66.4%). The majority of donors belonged to blood Group O (154; 38.6%), followed by Group B (142; 35.6%) and Group A (103; 25.8%). The age-wise distribution of the study population among the blood groups is presented in [Table/Fig-1].

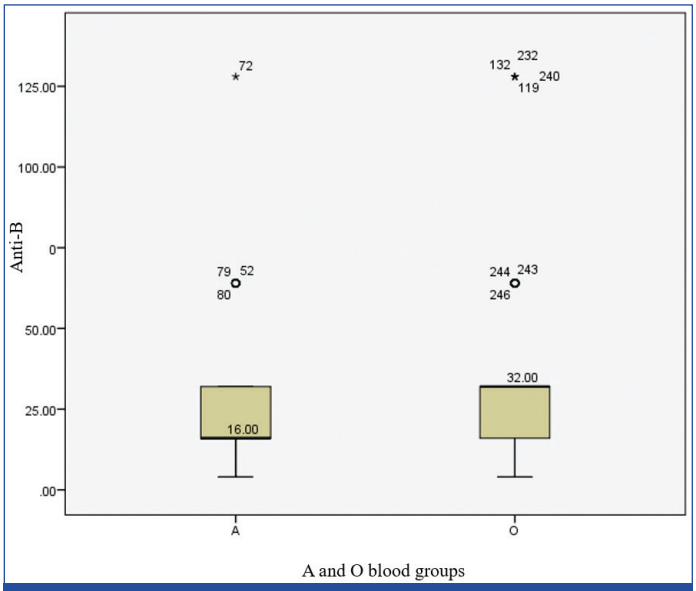
Age (years)	Blood group		
	'A' n (%)	'B' n (%)	'O' n (%)
18-30	73 (70.88)	91 (64.08)	101 (65.58)
31-40	23 (22.33)	35 (24.65)	39 (25.33)
41-50	5 (4.85)	13 (9.16)	11 (7.14)
51-60	2 (1.94)	3 (2.11)	3 (1.95)
Total	103 (100)	142 (100)	154 (100)

[Table/Fig-1]: Age-wise distribution of study population among the blood groups.

The distribution of median anti-A and anti-B titers in A, B, O blood groups is as follows: The median anti-A titer in blood groups B and O was 32 (IQR 8-64) and 32 (IQR 16-64), respectively, after considering outliers, as shown in [Table/Fig-2]. The median anti-B titer in blood groups A and O was 16 (IQR 8-32) and 32 (IQR 16-32), respectively, after considering outliers, as shown in [Table/Fig-3].



[Table/Fig-2]: Median anti-A titer in blood groups B and O.



[Table/Fig-3]: Median anti-B titer in blood groups A and O.

Age-wise distribution of anti-B titers in Group A: Most of the study population with titers <64 were in the age group of 18-30 years (62; 70.45%), and for titers >64, most of the study population were also in the same age group (11; 73.33%), as shown in [Table/Fig-4]. There was no statistically significant difference between the age groups and antibody titers, with a p-value of 0.425.

Age (years)	Anti-B titers in Group A n (%)			Median titers (IQR)
	<64	>64		
18-30	62 (70.45)	11 (73.33)	The Chi-square value is 2.789 with p-value 0.425	16 (16-32)
31-40	21 (23.86)	2 (13.33)		16 (8-32)
41-50	4 (4.55)	1 (6.67)		16 (8-16)
51-60	1 (1.14)	1 (6.67)		8 (8-8)
Total	88 (100)	15 (100)		

[Table/Fig-4]: Age-wise distribution of anti-B titers in group A.

Age-wise distribution of anti-A titers in Group B: Most of the study population with titers <64 were in the age group of 18-30 years (51; 54.84%), and for titers >64, most of the study population were also in the same age group (39; 79.59%), as shown in [Table/Fig-5]. A statistically significant difference between the age groups and antibody titers was observed, with a p-value of 0.007.

Age (years)	Anti-A titers in Group B n (%)			Median titers (IQR)
	<64	>64		
18-30	51 (54.84)	39 (79.59)	The Chi-square value is 12.254 with p-value 0.007	32 (32-64)
31-40	26 (27.96)	10 (20.41)		32 (16-64)
41-50	13 (13.98)	0		16 (16-32)
51-60	3 (3.22)	0		16 (4-16)
Total	93 (100)	49 (100)		

[Table/Fig-5]: Age-wise distribution of anti-A titers in group B.

Age-wise distribution of anti-A titers in blood Group O: Most of the study population with titers <64 were in the age group of 18-30 years (44; 54.32%), and for titers >64, most of the study population were also in the same age group (58; 79.45%), as shown in [Table/Fig-6]. A statistically significant difference between the age groups and antibody titers was observed, with a p-value of 0.002.

Age (years)	Anti-A titers in Group O n (%)			Median titers (IQR)
	<64	>64		
18-30	44 (54.32)	58 (79.45)	The Chi-square value is 14.540 with p-value 0.002	64 (32-80)
31-40	24 (29.63)	14 (19.18)		32 (32-64)
41-50	10 (12.35)	1 (1.37)		32 (16-32)
51-60	3 (3.70)	0		16 (16-16)
Total	81 (100)	73 (100)		

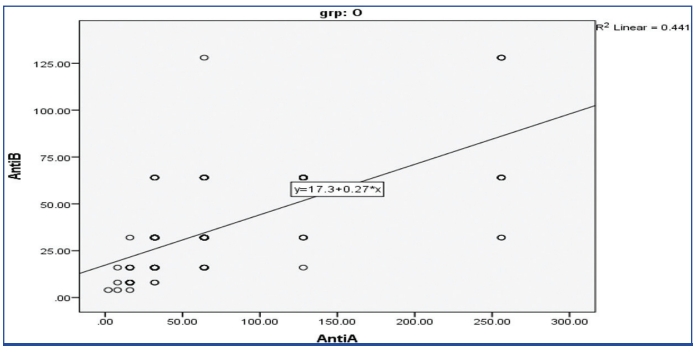
[Table/Fig-6]: Age-wise distribution of anti-A titers in blood group O.

Age-wise distribution of anti-B titers in blood Group O: Most of the study population with titers <64 were in the age group of 18-30 years (73; 60.83%), and for titers >64, most of the study population were also in the same age group (29; 85.29%), as shown in [Table/Fig-7]. A statistically significant difference between the age groups and antibody titers was observed, with a p-value of 0.044.

Age (years)	Anti-B titers in Group O n (%)			Median titers (IQR)
	<64	>64		
18-30	73 (60.83)	29 (85.29)	The Chi-square value is 8.117 with p-value 0.044	32 (28-64)
31-40	33 (27.5)	5 (14.71)		32 (16-32)
41-50	11 (9.17)	0		16 (8-32)
51-60	3 (2.5)	0		8 (8-8)
Total	120 (100)	34 (100)		

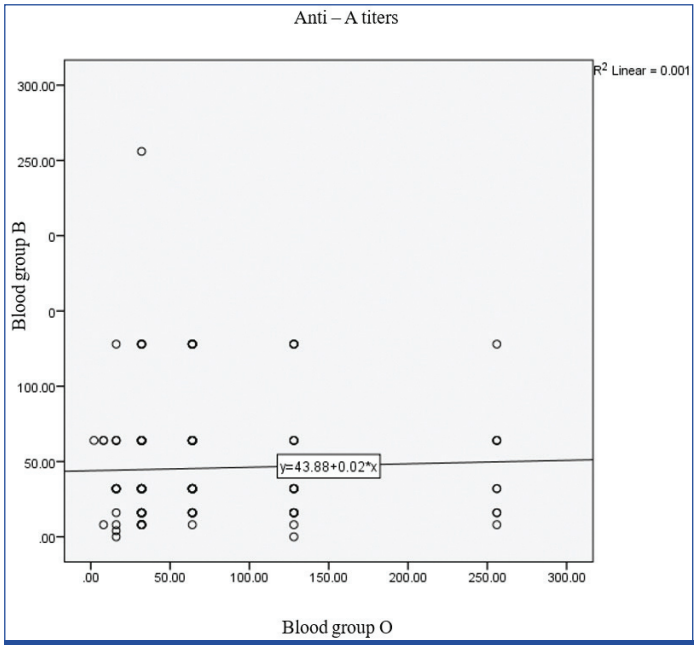
[Table/Fig-7]: Age-wise distribution of anti-B titers in blood Group O.

Correlation of titers in A, B, O blood groups: Anti-A and Anti-B titers in blood Group O were positively correlated, meaning that when anti-A titers were higher in an individual, their anti-B titers were also higher, with a Pearson correlation value of 0.664, which was statistically significant (p-value <0.001), as shown in [Table/Fig-8].

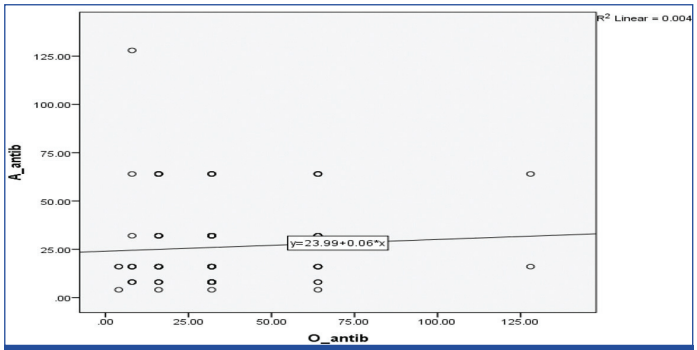


[Table/Fig-8]: Correlation between anti-A and anti-B in group O blood donors.

There was no correlation observed for anti-A titers between blood groups B and O donors, with a Pearson correlation value of 0.036, and there was no statistically significant difference (p-value=0.671), as shown in [Table/Fig-9]. Similarly, there was no correlation observed for anti-B titers between blood groups A and O donors, with a Pearson correlation value of 0.066, and there was no statistically significant difference (p-value=0.505), as shown in [Table/Fig-10].



[Table/Fig-9]: Correlation of anti-A between blood group B and O donors.



[Table/Fig-10]: Correlation of anti-B between blood group A and O donors.

DISCUSSION

The present study aimed to estimate the anti-A and anti-B titers in Group A, Group B, and Group O blood donors. Although many studies [22-24] have been conducted to assess the antibody titers in blood Group O, to the best of our knowledge, this was the first study from Southern India to assess the antibody titers across all blood groups, according to our literature search.

Distribution of IgM anti-A and anti-B Titers

The majority of the studies [18,19] have considered antibody titers of >64 as high titers, although different studies have used different critical titers [22,25]. Present study took 64 as the cut-off titer based on the aforementioned studies for the analysis. The proportion of blood Group A individuals with IgM anti-B titers of <64 was 88 (85.43%) and >64 was 15 (14.57%). For blood Group B individuals, <64 were 93 (65.5%) and >64 were 49 (34.5%). In blood Group O individuals, IgM anti-A titers of <64 were 81 (52.60%) and >64 were 73 (47.40%), whereas IgM anti-B titers of <64 were 120 (77.92%) and >64 were 34 (22.08%). Therefore, the proportion of donors with IgM anti-A titers of >64 was more common compared to those with IgM anti-B titers of >64. Kumar K et al., reported that IgM anti-B titers of <64 in Group A were seen in 92.86% and >64 in 7.14%, and IgM-A titers of <64 in Group B were seen in 87.71% and >64 in 12.29%, which was not in concordance with present study, as IgM

anti-A titers of >64 in blood Group B individuals were more common in present study [26].

Studies conducted by Hashim M et al., Bazigou F et al., Gopal S et al., Kumar K et al., and Kannan S et al., found that IgM anti-A titers in blood Group O of <64 were 63.9%, 44.28%, 59%, 64%, and 60.57%, respectively, and titers of >64 were 36.1%, 55.72%, 41%, 36%, and 39.43%, respectively, which were similar to present study [18,19,23,26,27]. França NDG de et al., considered 128 as a critical titer, with titers of <128 seen in 90.71% and >128 in 9.29%. According to them, the percentage of individuals with low titers was higher [28].

Similar to present study, a study conducted by Hashim M et al., observed that IgM anti-B titers in blood Group O individuals of <64 were seen in 73.4%, and >64 in 26.6% [18]. Bazigou F et al., showed that titers of <64 were seen in 52.85%, and >64 in 47.14%, which was concordant with present study [19]. Present study results were also consistent with other studies done by Gopal S et al., and Kumar K et al., Kannan S et al., who reported that titers of >64 were seen in 38%, 32%, and 36.86%, respectively [23,26,27].

Age-wise distribution of IgM titers in blood groups A and B: Among Group A individuals, IgM anti-B titers of <64 seen in the age group of 18-30 years were 62 (70.45%), followed by other age groups, and those having titers of >64 in the age group of 18-30 years were 11 (73.33%). This was consistent with the study conducted by Kumar K et al., [26]. Present study results were similar to those of Bazigou F et al., Sood R et al., and Thattanon P et al., who reported no association between age and antibody titers [19,22,29].

Among Group B individuals, IgM anti-A titers of <64 seen in the age group of 18-30 years were 51 (54.84%), and those having titers of >64 in the age group of 18-30 years were 39 (79.59%). The age distribution of titers in present study was similar to the study done by Kumar K et al., [26]. We found a statistically significant difference between age and IgM anti-A titers in blood Group B individuals (p-value=0.007), which shows that as the age increases, IgM anti-A titers decrease. Tendulkar AA et al., also showed an inverse relation between levels and age, with titer levels reducing as age progressed [24].

Age-wise distribution of titers in blood Group O: Among blood Group O, individuals having IgM anti-A titers of <64 in the age group of 18-30 years were 44 (54.32%), and those having titers of >64 in the age group of 18-30 years were 58 (79.45%). Present study reports are concordant with that of a study conducted by Kumar K et al., [26]. Present study found a statistically significant difference between age and IgM anti-A titers (p-value=0.002), which shows that as age increases, IgM anti-A titer decreases. The study population of blood Group O having IgM anti-B titers of <64 in the age group of 18-30 years were 73 (60.83%), and those having titers of >64 in the age group of 18-30 years were 29 (85.29%). Present study results were comparable to the study conducted in South Rajasthan [26]. Present study found a statistically significant difference between age and IgM anti-B titers (p-value=0.044), which shows that as age increases, IgM anti-B titer decreases.

In the present study, Group A individuals having median anti-B titers were similar until 50 years, then decreased which was not concordant to the study done by Datta SS et al., where titers were higher in the 40-49 years age group [30]. Whereas median anti-A in Group B donors and median anti-A and anti-B in Group O donors decreased as age increased, which was similar to Datta SS et al., [30].

Correlation of IgM anti-A and anti-B across the blood groups: There was no correlation observed for IgM anti-A antibodies between blood groups B and O donors, with a Pearson correlation value of 0.036 (p-value=0.671). Present study also found no correlation of IgM anti-B antibodies between blood groups A and O donors, with a Pearson correlation value of 0.066 (p-value=0.505).

However, present study observed a positive correlation between IgM anti-A and IgM anti-B antibodies in blood Group O, with a Pearson correlation value of 0.664, which was statistically significant (p-value <0.001). Hence, as the titer of anti-A increases, the titer of anti-B also increases in blood Group O individuals.

The comparison data of present study with other studies are tabulated in [Table/Fig-11] [24,26-28,30].

S. No.	Variable		Present study, 2024 (year of publication) Tirupati, Andhra Pradesh	Datta SS et al., Kolkata, 2021 [30]	Kannan S et al., JIPMER, 2020 [27]	Kumar K et al., Rajasthan, 2018 [26]	Tendulkar AA et al., Mumbai, 2017 [24]	França NDG de et al., Brazil, 2011 [28]
1.	Common age group		18-30 years (66%)	18-30 years (70%)	31-40 years (41%)	18-29 years (60%)	18-29 years (44%)	18-29 years (30%)
2.	Distribution of IgM Anti-A and anti-B in O group		IgM Anti-A high titers (>64) were more compared to anti-B titers	Not done	IgM anti-A high titers (>64) were more compared to anti-B titers	IgM anti-A high titers (>64) were more compared to anti-B titers	High titers in majority of study population	IgM anti-A high titers (>64) were more compared to anti-B titers
3.	Distribution of IgM anti-A and anti-B in groups B and A		High titers were more in B group than A group	Not done	Not done	High titers were more in B group than A group	Not done	Not done
4.	IgM antibody Median titer	Anti-B in Group A	16 (IQR 8-32)	24 (IQR8-64)	Not done	Not done	Not done	Not done
5.		Anti-A in Group B	32 (IQR 8-64)	16 (IQR 8-16)	Not done	Not done	Not done	Not done
6.		Anti-A in Group O	32 (IQR 16-64)	32 (IQR 16-64)	64 (IQR 16-64)	78 (IQR 2-256)	110 (IQR 4-1024)	32 (IQR 16-64)
7.		Anti-B in Group O	32 (IQR 16-32)	48 (IQR 32-64)	32 (IQR 8-64)	64 (IQR 2-256)	96 (IQR 4-2048)	16 (IQR 8-32)
8.	Median IgM antibody titers across age groups	Anti-B in Group A	Same (16) up to 50 years, later decreased	More in 40-49 years age group	Not done	Not done	Not done	Not done
9.		Anti-A in Group B	As age increased median titer decreases	As age increased median titer decreases	Not done	Not done	Not done	Not done
10.		Anti-A and anti-B titer in Group O	As age increased both median titers were decreased	As age increased both median titers were decreased	As age increased both median titers were decreased	As age increased both median titers were decreased	As age increased both median titers were decreased	As age increased both median titers were decreased

[Table/Fig-11]: Comparison data of present study with other studies [24,26-28,30].

Limitation(s)

Though the sample size was adequate as per the calculation, present study could not include a larger number of female populations. Hence, authors could not assess the difference of antibody titrations among the genders.

Present study did not conduct antibody titrations of IgG antibody using dithiothreitol treatment, as the method was not standardised in our laboratory. Although the test using conventional tube technology was performed, which is considered the gold standard, it has its own limitations of subjective interpretation.

CONCLUSION(S)

The database should be maintained in all institutes by estimating antibody titers for every A, B, O blood group donors. Group O whole blood or platelets from donors of age >40 years can be selected and transfused to non Group O individuals as the titer of IgM anti-A and IgM anti-B decreases with age. However, blood from donors having a titer value of >64 should be given to group-specific recipients only. In case of non availability of group-specific platelets and in emergency situations, Group A platelets may be transfused across the ABO barrier even without performing anti-A and anti-B titers, since 85% of Group A donors had IgM anti-B titers <64.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Sep 28, 2023
- Manual Googling: Dec 02, 2023
- iThenticate Software: Dec 11, 2023 (15%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Sep 28, 2023**
Date of Peer Review: **Nov 18, 2023**
Date of Acceptance: **Dec 14, 2023**
Date of Publishing: **Feb 01, 2024**