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Research Article

ABO ANTIBODY TITER STUDY IN HEALTHY DONORS IN TERTIARY CARE CENTRE OF SOUTHERN RAJASTHAN

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A B S T R A C T

Introduction-Besides risk of TTIs (transfusion transmitted diseases), plasma containing product like platelets, fresh frozen plasma, whole blood and IVIg also have naturally occurring ABO antibodies and sometimes these products are used ABO-incompatible. These ABO-antibodies sometimes can cause hemolytic reaction in recipients. There is no generalised consensus on critical titer or high titer level of ABO antibodies in incompatible units (which can cause haemolysis) on national level. Generally > 64 is considered as high titer for IgM and >256 for IgG antibodies.

AIMS AND OBJECTIVES- Our aims were to evaluate the trends ofanti-A and anti-B antibody titer levels in healthy blood donors, in relation to age and gender, in relation to different ABO blood group and to define a strategy to deal with incompatible ABO plasma containing products.

Material and Methods-We used conventional tube method of titrationas per AABB standard, to identify level of ABO-IgM and IgG antibodies in randomly selected 1050 healthy blood donors.

Result- Our study shows O blood group has much high titer of all type of ABO antibodies than A and B groups. Which was statically highly significant (p value <0.001).

In "O +ve" blood donors, % of donors having anti-A & anti-B IgM antibody titer >64 were as high as 39.42% & 36.85% respectively. And % of donors having anti-A & anti-B IgG antibody titer >256 were as high as 41.71%. & 41.42% respectively. The distribution was statistically significant for anti-A IgG & IgM antibodies both (p value 0.027 & 0.023) respectively. Females had higher HT (high titer) anti-B antibody than males in 'O' blood group but the distribution was statically non significant (p value 0.08 & 0.10 for IgM & IgG respectively).

Conclusions-It is important to implement a practical strategy by Transfusion Medicine speciality to reduce the risk of haemolysis caused by blood products which contain ABO incompatible plasma. This study concludes that titration of ABO antibodies in blood banks will increase safety in transfusion of products, containing ABO incompatible plasma like platelets, FFPs, WB(whole blood) and IVIg.

INTRODUCTION

Blood transfusion constitutes an important part of various treatment protocols. Blood must be transfused keeping in mind certain precautions because like drugs, blood and its components also have the propensity to cause side effect such as introduction of donor antigens and antibodies in the recipient, transfusion reaction or exposure to various transfusion transmissible disease and it is very important for clinicians to be aware of these potential risks to the recipient of blood and its products. Hence, periodic review of blood component usage is essential in any hospital or health set-up.

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The decision to transfuse a hospitalized patient must balance the known risks of transfusion with appropriate utilization of blood as a scarce resource.

Besides risk of TTIs, Plasma containing product like platelets, FFP and IVIg also have naturally occurring ABO antibodies and sometimes these products are used ABO-incompatible & can result in haemolytic transfusion reaction. Contributing risk factors include the small blood volume of the patient, exposure to a large cumulative volume of incompatible plasma over time, and high-titer anti-A, anti-B or both in donor plasma (Fung MK *et al*.,2007 & Cooling L, 2007).

The ABO system is the only blood group system in which individuals have antibodies in their serum to antigens that are absent from their RBCs ie. anti-A in blood group B, anti-B in blood group A, anti-A and anti-B in O blood group and no

antibodies in AB blood group individuals. This occurs without any exposure to RBCs through transfusion or pregnancy.

Anti-A and anti-B molecules may be IgM, IgG or IgA. Some sera contain all three classes and non-stimulated individuals are predominantly IgM. Changes in the characteristics of anti-A or anti-B occur as a result of further immunization with pregnancy or by incompatible transfusions. They are serologically detectable through increases in titers, agglutinin avidity and haemolytic activity and have greater activity at 37ºC (Daniels G, 2002).

Platelets inventory have very short life span of 5 days and O blood group donor are much more common than other ABO group donors in India. So it is not uncommon to issue ABO-incompatible (minor mismatched) group SDP and RDP. But these units are seldom admitted for antibody titration.

Acute haemolysis has been reported following transfusion of non-group specific platelets (specially single donor platelets) and may be more common than is appreciated. Random donor platelets from group O donors have also been implicated in these reactions (Fung MK *et al*., 2007). Because RDPs (usually 4-6 units or pooled) issued for adult have 200-400 ml plasma and in children, there is already less red cell mass.

'O' group whole blood for exchange transfusion is also common practice and WB units may contain minor cross match incompatible antibodies. In newborn, these antibodies may destroy newly forming RBCs. 'O' group whole blood transfusion is also common in trauma patient in military settings and where components are not available.

Hence the transfusion of group O plasma to other blood group recipients has been a matter of debate and discussion.

We were not doing antibody titer for ABO antibodies for platelets or plasma product in routine at our centre before this study.

There is no generalised consensus on critical titer or high titer level of ABO antibodies in incompatible plalelets units on national level. Generally $\geq$64 is considered as high titer for IgM and $\geq$256 for IgG antibodies.

Chow *et al*. (1991) advocate using only apheresis platelets with isoagglutinin titers less than 1:32, and Reis and Coovadia (1989) recommend removal of donor plasma when titers are 1:64 or higher.

Case reports from different workers have tried to report the titer levels which may be considered dangerous (Josephson CD *et al*., 2004 & Karen Quillen *et al*, 2011).

Thus transfusion should be considered in terms of risk versus benefit. It should be planned judiciously and through adopting the guidelines of safe blood transfusion. The decision to transfuse should be individualized, based on a rational approach.

Titration of ABO antibodies in blood banks will increase safety in non-identical ABO transfusions of plasma containing products like platelets, FFP, WB. The Transfusion Medicine speciality should consider measures to increase ABO-identical PLT transfusions and physicians should be aware of potential adverse outcomes when transfusing non-identical ABO PLTs. Although most cases recover with appropriate supportive care, reactions can be fatal.

Aims and Objectives

1. To evaluate the trends of anti-A and anti-B antibody titer levels in healthy blood donors.
2. To evaluate the trends of anti-A and anti-B antibody titer levels in relation to age and gender.
3. To evaluate the trends of anti-A and anti-B antibody titer levels in relation to different ABO blood group.
4. To define a strategy for routine antibody titration for incompatible ABO plasma containing products.

MATERIAL AND METHODS

This prospective study carried out at the Department of Transfusion Medicine, Blood Bank, MBGH, R.N.T. Medical College Udaipur during the periods of July 2016 to Dec 2017 in 1050 healthy donors for ABO antibody titration. Donors were selected randomly. We used IgM titer 64 and IgG titer 256 as a critical titer for our study. The critical titer value of 64 and 256 was selected based on cited literature (Josephson CD *et al*., 2004 & Karen Quillen *et al*, 2011).

Inclusion criteria

1. Healthy blood donors of A , B and O blood group
2. Donors of 18 to 60 yrs age of both sex group included

Exclusion criteria

1. AB blood group donors
2. Rh negative donors

For antibody titration, conventional tube method was used as per AABB standard (AABB TECHNICAL MANUAL 17th edition).

Titration is a semiquantitative method used to determine the concentration of antibody in a serum sample. We used this method to identify level of ABO-IgM and IgG antibodies in healthy blood donors. IgM antibodies react at RT, while IgG antibodies react at AHG phase and 37ºC .

Specimen: Plasma of healthy donors to be titrated. (samples were taken from donor unit i.e. WB, FFP, RDP)

Reagents

1. Red cells that express the antigen(s) corresponding to the antibody specificity(ies), in a 3% saline suspension
2. Normal Saline (0.9%)
3. AHG (anti IgG)

Procedure steps

First master dilutions were prepared.

Steps to prepare master dilution

- 10 test tubes were labeled according to the serum dilution (eg, 1:1, 1:2, 1:4,…….1:512). A 1:1 dilution means one volume of serum undiluted; a 1:2 dilution means one volume of serum in a final volume of two, or a 50% solution of serum in the diluent.
- One volume (200 µl) of saline delivered to all test tubes except the first (undiluted, 1:1) tube.
- An equal volume (200 µl) of serum added to each of the first two tubes (undiluted and 1:2).
- Using a clean pipette, contents of the 1:2 dilution mixed several times, and one volume (200 µl) transferred into the next tube (the 1:4 dilution). Same process continued

for all dilutions, using a clean pipette to mix and transfer each dilution.

- One volume of diluted serum removed from the final tube, and saved for use if further dilutions were required. Thus master dilutions were prepared.

Next steps for IgM antibodies titration

- Now 10 tubes were labeled for the appropriate dilutions (eg, 1:1, 1:2, 1:4,…….1:512). One set for A blood group samples (for anti-B titration), One set for B blood group samples (for anti-A titration) and two set for O blood group sample (one set to identify anti-A and another set for anti-B).
- Using separate pipettes for each dilution, 100 µl of each diluted serum from master dilution, transferred into the corresponding labeled tubes, 50 µl of a 3% suspension of appropriate red cells were added in these tubes (A group red cell to identify anti-A and B group red cells to identify anti-B).
- The tubes were mixed well and incubated for 15 min at room temperature and centrifuged for reading at 1000 rpm for one minute. Button was gently dislodged, test result was examined macroscopically for IgM antibody ; the reaction was read for grading and result was recorded.

Next steps for IgG antibodies titration

- Like IgM, Separate sets of appropriately labeled (eg, 1:1, 1:2, 1:4,…….1:512.) test tubes were prepared from master dilution for IgG antibody titration, 50 µl of a 3% suspension of appropriate red cells were added in these tubes (A group red cell to identify anti-A and B group red cells to identify anti-B).
- Tubes were incubated at 37^0C for 60 minutes, saline washed 3 times after incubation.
- Decanting after last wash, one drop AHG reagent was added, and centrifuged at 1000 rpm for 1 min.
- Button was gently dislodged, test results were examined macroscopically for IgG antibodies; the reaction was read for grading and result was recorded.
- Results were entered in Microsoft excel sheet for master chart same time.

Interpretation

1. Titer is the reciprocal of the highest dilution that produces 1+ Macroscopic agglutination. The titer is reported as the reciprocal of the dilution level (e.g, 32 - *for* 1 in 32 or 1:32).
2. If there is agglutination in the tube containing the most dilute serum, the endpoint has not been reached, and additional dilutions should be prepared and tested.

OBSERVATION AND RESULTS

- Total 1050 donors, 300 male and 50 female donors of each blood group of A, B and O were taken in the study.
- 60% of all 'A' blood group donors, 45.43% of all 'O' group donors and 43.43% of all 'B' blood group donors were in the age group of 18-29 yrs, i.e. from the young age group were the highest donors.
- Our study shows O blood group has much high titer of all type of ABO antibodies than A and B groups. Which was statically also highly significant (p value <0.001).
- In "B+ve" blood donors, only 12.28% had anti-A IgM antibody titer ≥64. Male and female donors with the IgM titer level ≥64 were 12.66% and 10% respectively, which was almost equal. The anti-A IgM antibody titers ranged from 2-256 and 2-128 in males and females respectively. Only 0.86% had anti-A IgG titer ≥ 256. Male and female donors with IgG titer level ≥256 were 0.66% and 2% respectively, which was almost equal. The distribution of HT (high titer) anti-A IgM & IgG in B blood group male and female was statically non-significant (p value was 0.595 & 0.344 respectively).
- In "A+ve" blood donors, % of donors having anti B IgM antibody titer ≥64 was 7.14%. In males and females, % of donors having anti-B IgM antibody titer level ≥64 was 7.33% and 6% respectively, which was quite equal. Percentage of donors having anti-B IgG antibody titer ≥256 was as low as 0.29%. Percentage of donors with anti-B IgG titer level ≥256 was none in males and only 2% in females. This distribution was statistically significant in IgG antibodies (p value 0.014) & non significant for IgM antibodies (p value 0.735).
- In "O +ve" blood donors, % of donors having anti-A IgM antibody titer ≥64 was as high as 39.42%. In males and females, donors with anti-A IgM titer ≥64 were 37% and 54% respectively. % of donors having anti-A IgG antibody titer ≥256 was as high as 41.71%. In males and females, donors with anti-A IgG titer ≥256 were 39.4% and 56% respectively. The distribution was statistically significant for anti-A IgG & IgM antibodies both (p value 0.027 & 0.023 respectively). This shows that HT anti-A antibody in 'O' group donors was more in female donors than in males.
- In "O +ve" blood donors, 36.85% donors had anti-B IgM antibody titer ≥64. In males and females, donors with the anti-B IgM titer level ≥64 were 35% and 48% respectively, which shows higher antibody titer in females over males. 41.42% donors had anti-B IgG antibody titer ≥256. In males and females, donors with anti-B IgG titer level ≥256 were 39.66% and 52% respectively. Females had higher HT anti-B antibody than males in 'O' blood group but the distribution was statically non significant (p value 0.08 & 0.10 for IgM & IgG respectively).
- There was high IgM anti-A antibody ranged 2 to 1024 (in male 2-1024 & female 4-1024) in 'O' blood group as compared B blood group ranged 2 to 256(M; 2-256 & F; 2-128).
- There was high IgM anti-B antibody ranged 2 to 1024 (M; 2-1024 & F; 4-512) in 'O' blood group as compared A blood group ranged 2 to 128(M; 2-128 & F; 2-128).
- There was high IgG anti-A antibody ranged 2 to 2048(M; 2-1024 & F; 8-2048) in 'O' blood group as compared B blood group ranged 2 to 512 (M; 2-512 & F; 2-256).
- There was high IgG anti-B antibody ranged 8 to 2048(M; 8-2048 & F; 16-1024) in 'O' blood group as compared A blood group ranged 2 to 256(M; 2-128 & F; 2-256).

- Mean titer level for anti-A & anti-B IgM antibodies in O group donors were 78.12 (2 to 1024) & 64.86(2 to 1024).
- Mean titer level for anti-A & anti-B IgG antibodies in O group donors were 199.63 (2 to 2048) & 199.98(8 to 2048).
- Titer level 8 for IgG & IgM antibodies was observed in maximum number of donors among the blood group A and B.
- 'O' blood group donors having anti-A and anti-B IgG antibody titers ≥512 were 14.29% and 13.14% respectively which was also considerable with CTT (conventional test tube) method.
- 'O' blood group donors having anti-A and anti-B IgM antibody titers ≥128 were 20.86% and 14.85% respectively which was also considerable with CTT.
- Percentage of donors with IgM antibody ≥64 were higher in age group 30-44 years for all ABO blood group.
- Percentage of donors with IgG antibody ≥256 were higher in age group 18-29 years in 'O' blood group and in age group 30-44 in A & B blood group.
- There was slight decrease in IgM & IgG antibodies in 'O' group in the age group of 45-60 years. But the distribution was statically significant for anti-A IgM & anti-B IgG (p value<0.001& 0.020).

Table No. 1 Distribution of Anti-A According to Gender in 'B' Blood Group Donors

Antibody	Titer	Male (n=300)	Female (n=50)	Total (n=350)
IgM	<64	262 (87.33%)	45 (90%)	307 (87.71%)
	≥64	38 (12.66%)	5 (10%)	43 (12.28%)
	Range	2-256	2-128	2-256
	P value	0.595 (NS)		
IgG	<256	298 (99.33%)	49 (98%)	347 (99.14%)
	≥256	2 (0.66%)	1 (2%)	3 (0.86%)
	Range	2-512	2-256	2-512
	P value	0.344 (NS)		

Table No.2 Distribution of Anti-B According to Gender in 'A' Blood Group Donors

Antibody	Titer	Male (n=300)	Female (n=50)	Total (n=350)
IgM	<64	278 (92.66%)	47 (94%)	325 (92.86%)
	≥64	22 (7.33%)	3 (6%)	25 (7.14%)
	Range	2-128	2-128	2-128
	p value	0.735 (NS)		
IgG	<256	300 (100%)	49 (98%)	349 (99.71%)
	≥256	0	1 (2%)	1 (0.29%)
	Range	2-128	2-256	2-256
	p value	0.014 (S)		

Table No. 3 Distribution of Anti-A According to Gender in 'O' Blood Group Donors

Antibody	Titer	Male (n=300)	Female (n=50)	Total (n=350)
IgM	<64	189 (63%)	23 (46%)	212 (60.57%)
	≥64	111 (37%)	27 (54%)	138 (39.42%)
	Range	2-1024	4-1024	2-1024
	P value	0.023 (S)		
IgG	<256	182 (6 0.66%)	22 (44%)	204 (58.28%)
	≥256	118 (39.4%)	28 (56%)	146 (41.71%)
	Range	2-2048	8-2048	2-2048
	P value	0.027 (S)		

Table No. 4 Distribution of Anti-B According to Gender in 'O' Blood Group Donors

Antibody	Titer	Male (n=300)	Female (n=50)	Total (n=350)
IgM	<64	195 (65%)	26 (52%)	221 (63.14%)
	≥64	105 (35%)	24 (48%)	129 (36.85%)
	Range	2-1024	4-512	2-1024
	P value	0.08 (NS)		
IgG	<256	181 (60.33%)	24 (48%)	205 (58.57%)
	≥256	119 (39.66%)	26 (52%)	145 (41.42%)
	Range	8-2048	16-1024	8-2048
	P value	0.10 (NS)		

Table No. 5 Distribution of IgG Antibody According to Blood Group (n=350)

Titer	Group 'A' Anti-B		Group 'B' Anti-A		Group 'O' Anti-A		Group 'O' Anti-B	
	No.	%	No.	%	No.	%	No.	%
2	51	14.57%	46	13.14%	4	1.14%	-	-
4	84	24.00%	80	22.86%	6	1.71%	-	-
8	114	32.57%	101	28.86%	16	4.57%	21	6.00%
16	70	20.00%	71	20.29%	45	12.86%	50	14.29%
32	25	7.14%	37	10.57%	50	14.29%	47	13.43%
64	4	1.14%	8	2.29%	49	14.00%	51	14.57%
128	1	0.29%	4	1.14%	34	9.71%	36	10.29%
256	1	0.29%	2	0.57%	96	27.43%	99	28.29%
512	-	-	1	0.29%	37	10.57%	33	9.43%
1024	-	-	-	-	10	2.86%	9	2.57%
2048	-	-	-	-	3	0.86%	4	1.14%

Table No.6 Distribution of IgM Antibody According to Blood Group (n=350)

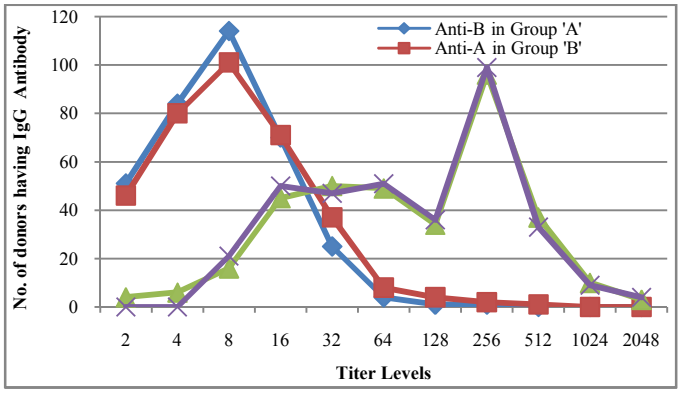
Titer	Group 'A' Anti-B		Group 'B' Anti-A		Group 'O' Anti-A		Group 'O' Anti-B	
	No.	%	No.	%	No.	%	No.	%
2	21	6.00%	18	5.14%	15	4.29%	10	2.86%
4	52	14.86%	49	14.00%	28	8.00%	42	12.00%
8	91	26.00%	85	24.29%	48	13.71%	40	11.43%
16	117	33.43%	104	29.71%	48	13.71%	59	16.86%
32	44	12.57%	51	14.57%	74	21.14%	70	20.00%
64	23	6.57%	38	10.86%	64	18.29%	77	22.00%
128	2	0.57%	4	1.14%	45	12.86%	32	9.14%
256	-	-	1	0.29%	14	4.00%	9	2.57%
512	-	-	-	-	8	2.29%	7	2.00%
1024	-	-	-	-	6	1.71%	4	1.14%

Table No.7 Distribution of IgM Titer ≥64 According Age and Blood Group

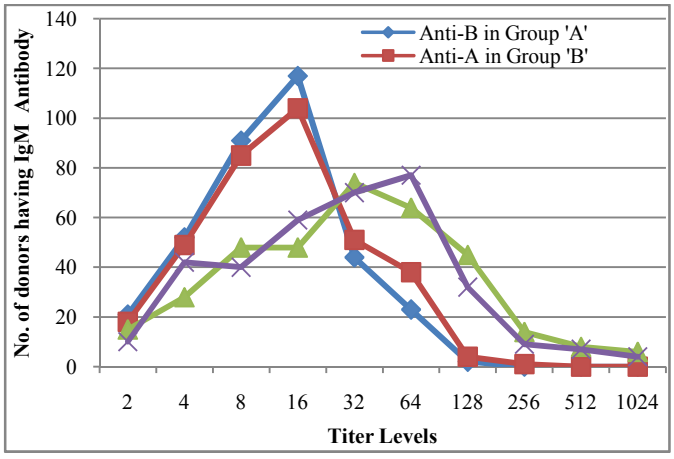
Age group (yrs)		Group 'A' Anti-B	Group 'B' Anti-A	Group 'O' Anti-A	Group 'O' Anti-B
18-29	Total donors	210	152	159	159
	Donors with Titer ≥64	15	16	64	63
	%	7.14%	10.53%	40.25%	39.62%
30-44	Total donors	100	136	155	155
	Donors with Titer ≥64	10	25	66	56
	%	10.00%	18.38%	42.58%	36.13%
45-60	Total donors	40	62	36	36
	Donors with Titer ≥64	-	2	7	10
	%	-	3.23%	19.44%	27.78%

Table No.8 Distribution of IgG Titer ≥256 According Age and Blood Group

Age group (yrs)		Group 'A'	Group 'B'	Group 'O'	Group 'O'
		Anti-B	Anti-A	Anti-A	Anti-B
18-29	Total donors	210	152	159	159
	Donors with Titer ≥256	-	1	73	72
	%	-	0.66%	45.91%	45.28%
30-44	Total donors	100	136	155	155
	Donors with Titer ≥256	1	2	62	70
	%	1.00%	1.47%	40.00%	45.16%
45-60	Total donors	40	62	36	36
	Donors with Titer ≥256	-	-	10	4
	%	-	-	27.78%	11.11%



Graph 1 Distribution of IgG Antibody According to Blood Group



Graph 2 Distribution of IgM Antibody According to Blood Group

DISCUSSION

The AABB Standards (Standards for Blood Banks and Transfusion Services; AABB, 2009) state that ‘‘the transfusion service shall have a policy concerning transfusion of components containing significant amounts of incompatible ABO antibodies or unexpected red cell antibodies.’ Therefore, screening for high titer of IgM and IgG (anti-A and Anti-B) is suggested when using platelets containing ABO incompatible plasma as significant number of donors found having HT (high titer) ABO antibodies.

In our study, in "O +ve" blood donors, % of donors having IgM ≥64 for anti-A antibody was as high as 39.42%, and for anti-B IgM it was 36.85%. And % of "O +ve" blood donors having IgG ≥256 for anti-A antibody was as high as 41.71%.and for anti-B IgG it was 41.42%.

Josephson (2004) *et al*. found 28 and 39 percent of samples (Group O apheresis donors) having critically high anti-A/A,B IgM and IgG titer , respectively using gel CAT (high titer criteria ≥64 for IgM and ≥256 for IgG). Haemolytic anti-A and anti-B was found in 25.1% and 14.9% donor samples respectively by Oyedeji OA *et al*. (2014). Bazigou F *et al*. (2015) found ‘O’ group APs (apheresis) donors having “high” anti-A and anti-B were relatively high, 55.8% and 47.2% respectively using gel card method assuming 64 as a high titer for IgM antibody. The anti-A and anti-B were ≥64 in 79.9% and 76.33% of all ‘O’ group donors, respectively in a study by Tendulkar AA *et al*. (2017), done at Tata Memorial Hospital but they used microplate method. Thus almost all studies shows considerable percentage of O group donors with high titer, though they used different method of titration and considered different level as dangerous level.

'O' blood group donors having anti-A and anti-B IgM antibodies titer ≥128 were 20.86% and 14.85% respectively in our study. Which was also significant with CTT method. At the dilution of 1 in 150, half of group O components were labeled as high by Karen Quillen *et al*. (2011) using gel card method. De França ND (2011) found group O donors having IgM anti-A > 128 = 9.29% and IgM anti-B > 128 = 4.81% in Brazil using conventional tube technique at room temperature. The anti-A and anti-B were ≥128 in 57.12% and 51.19% of all group O donors, respectively in a study by Tendulkar AA *et al* (2017) using microplate method. Thus if ≥128 is considered as HT, there are also considerable numbers of dangerous 'O' blood group donors.

In our study, in males and females, O blood group donors with anti-A IgM ≥64 were 37% and 54% respectively. In males and females, donors with anti-B IgM ≥64 level were 35% and 48% respectively. In males and females, donors with anti-A IgG ≥256 were 39.4% and 56% respectively. In males and females, donors with anti-B IgG level ≥256 were 39.66% and 52% respectively. So our study showed high titer ABO antibodies (both anti-A and anti-B) significantly higher in female than male. This distribution was statistically significant ($p<0.05$) for anti-A IgM and IgG. Phatchira Thattanon *et al*. (2015) and Sood R *et al*. (2016) showed no association between anti-A and anti-B titer with age and gender in blood donors. While Mariana Martins Godin *et al* (2016) found that frequency among women was higher than that of men. Tendulkar AA *et al*. (2017) showed that female donor's anti-A and anti-B titer were significantly higher when compared to males which is similar to our study.

Mean titer level for anti-A & anti-B IgM antibodies in O group donors were 78.12 (2 to 1024) & 64.86 (2 to 1024). Mean titer level for anti-A & anti-B IgG antibodies in O group donors were 199.63 (2 to 2048) & 199.98 (8 to 2048) in our study. De França ND *et al*. (2011) showed ABO antibodies titer ranged from 1 to 2048 at RT. Eun Su Park *et al*. (2014) found median values of anti-B antibody titer in blood group O obtained using IS and AHG tubes were 16 (4- 128) and 32 (4-256), and those of anti-A antibody titer in blood group O were 16 (4-128) and 32 (8-256), respectively. But they included only 60 ‘O’ group donors. Phatchira Thattanon *et al*. (2015) showed IgG anti-A titer obtained by IAT-CTT were 128 (4-4096) and IgG anti-B titer were 192 (1-2048), in group O

donors respectively. Anti-A titer ranged from 2 to 1024 (mean: 64), while anti-B titer ranged from 2 to 256 (mean: 32) in a study by Bazigou F *et al*. (2015). Sood R *et al*. (2016) in a study done at Saket City Hospital, New Delhi, found Mean anti-A and anti-B titer in group O plasma were, respectively, 163.28, 113.42 for IgM antibody and 174.50, 152.98 for IgG antibody by tube method in O group donors. In our study these data were 78.12, 64.86 for IgM and 199.63, 199.98 for IgG antibody in O group donors. Tendulkar AA *et al*. (2017) found titer in O group donors ranging from 4 to 1024 for anti-A and 4 to 2048 for anti-B IgM.

Mean values and % of high titer donors were different in various studies. This may be due to different sample size, sample selection, different titration method and donors from different environment. According to Mazda T *et al*. (2007) ABO antibody levels depend on the ethnic background and environmental factors and Kumlien G *et al*. (2007) found that for antibodies titration, results for the same sample differed by a median of three (range 0 to 6) steps by different method.

There was IgM anti-A antibody ranged in B blood group ranged 2 to 256(M; 2-256 & F; 2-128) and IgG anti-A antibody ranged 2 to 512 (M; 2-512 & F; 2-256). There was IgM anti-B antibody ranged in A blood group ranged 2 to 128 (M; 2-128 & F; 2-128) and IgG anti-B antibody ranged 2 to 256 (M; 2-128 & F; 2-256). Level 8 for IgG & IgM antibodies was observed in maximum number of donors among the blood group A and B in our study. In a study by Eun Su Park *et al*. (2014) the median values of anti-B titer in blood group A obtained using IS and AHG tube methods were 16 (2- 256) and 8 (1-512), respectively, and those of anti-A titer in blood group B obtained using IS and AHG tube methods were 16 (2-128) and 8 (2-64), respectively. Both values were higher in IS than in AHG tube methods similar to our study.

In "B +ve" blood donors, 12.28% had anti-A IgM antibody $\geq$64. In "A +ve" blood donors, % of donors having anti-B IgM antibody $\geq$64 was 7.14% while donor with HT IgG antibodies were negligible which is quite considerable for apheresis donors. So ABO mismatched A & B group apheresis donors should also go for HT IgM antibody screening.

IgM levels were high in A and B blood group donors while IgG levels were more in O group donors similarly with other studies.

Percentage of 'O' group donors with high titer of anti-A was more than anti-B (specially in female) .which was also confirmed by Oyedeji OA *et al*. (2014), Phatchira Thattanon *et al*. (2015), Bazigou F *et al*. (2015), Sood R *et al*. (2016) and Tendulkar AA *et al*. (2017)

Different studies showed different opinion about relationship between age and ABO antibody titers. As Phatchira Thattanon *et al*. (2015), Bazigou F *et al*. (2015) and Sood R *et al*. (2016) showed no association between age and ABO antibody titers. While Tendulkar AA *et al*. (2017) showed an inverse relation between levels and age (males: anti-A and anti-B, females: anti-B) with levels reducing as age progressed. De França ND (2011) showed low mean titer for both anti-A and anti-B antibodies in over 50-year-old men. We divided donors in 3 age groups (18-29; 30-44 and 45-60 years) for study but found no direct relationship between age and antibodies titer. There was slight decrease in IgM & IgG antibodies in 'O' group in the age group of 45-60 years. But the distribution was statically significant for anti-A IgM & anti-B IgG (p value <0.001 & 0.020). Percentage of donors with IgM antibody $\geq$64 were higher in age group 30-44 years for all ABO blood group. Percentage of donors with IgG antibody $\geq$256 was higher in age group 18-29 years in 'O' blood group and in age group 30-44 in A & B blood group.

CONCLUSION

This study concludes that titration of ABO antibodies in blood banks will increase safety in transfusion of products, containing ABO incompatible plasma like platelets, FFPs, WB. So it is important to implement a practical strategy by Transfusion Medicine speciality to reduce the risk of haemolysis caused by blood products which contain ABO incompatible plasma. Studies showed haemolysis in such incompatible transfusions highlight the significance of titer levels. Although most cases recover with appropriate supportive care, reactions can be fatal. Additionally, ABO antibody titration is important especially in cases of ABO-HDFN and ABO incompatible stem cell and solid organ transplantations. In the absence of a global consensus on this topic, institutes may need to formulate their own guidelines on handling the products, containing ABO incompatible plasma. Various policies are being excercised for preventing haemolytic reactions from transfusion of products containing ABO incompatible plasma ie; volume reduction of plasma, limiting the daily amount of ABO-incompatible plasma in platelets transfused, pooling of whole-blood derived platelet concentrates, use of platelet additive solution, incompatible plasma is replaced by AB plasma, The platelets are washed and resuspended in saline. Most of above methods require manipulation of platelet products, may decrease product's expiry to 4 hrs and/or may decrease platelet yield, there is an alternative approach to determining the titer of anti-A or anti-B in group O platelets before transfusion.

Recommendation for Making Policy To Reduce The Risk Of Haemolysis Caused By Transfusion Of Products, Containing ABO Incompatible Plasma

1. Clinicians should be made aware of potential adverse outcomes of transfusion of products, containing ABO incompatible plasma.
2. First priority should always be given to group specific platelets unit for transfusion. ABO incompatible platelets (specially O group platelets) transfusion should be the last choice, specially in paediatric patients.
3. If apheresis unit is to be given plasma incompatible. Always do ABO antibody titer of otherwise healthy donor prior to procedure.
4. The use of platelets from A donors for B recipients, and vice versa, is preferred over the use of O donors for recipients of another ABO blood group.
5. For RDPs, all O group units should be tested for ABO antibody titer, if are to be given for other group recipient and should only be issued if having IgM titer <64 and IgG < 256. Always try to keep four units of 'O' group RDPs in stock for which antibody titration has been done and are of low titer for ABO antibodies for emergency cases. A, B or AB group RDPs can be given with 'O' group units to add a layer of safety.

6. If a unit is found to be of high titer, it should be mentioned on that particular unit. This unit should be kept for ABO identical or 'O' group recipients only.
7. For out of group platelets or FFP, preference should be given according to AB>A>B>O.
8. 'O' blood group units (WB) with low titers should be issued for exsanguinous transfusion or 'O' PRBC with AB plasma should be used.

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