Short Communication

Anti-A and Anti-B Titer Among Blood Group O Donors at a Tertiary Care Centre Lahore, Pakistan

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ABSTRACT

The aim of this study was to evaluate the prevalence of IgM and IgG type anti-A and anti-B antibodies and the titer of these antibodies among blood group O donors at a tertiary care hospital. This is a cross-sectional study conducted at the department of Hematology and Transfusion Medicine in Children's Hospital and the Institute of Child Health, Lahore, Pakistan over a period of four months from Oct, 2019 to Feb, 2020. Blood samples were collected from 350 healthy and voluntary group "O" donors (males = 320 and females = 30, median age 27 years ± 6.4 SD)**.** Estimation of IgM type anti-A and anti-B titers was done using the standard tube technique and the samples positive for hemolysis were further evaluated for titers of IgG type anti-A and anti-B. All the titers were confirmed microscopically. The total prevalence of IgG anti-A and anti-B hemolysins was 15.9%. The most frequently observed IgM anti-A and anti-B titers were 64 in 29.4% (n = 103) and 32 in 24.9% (n = 87) donor samples, respectively. IgG type anti-A and anti-B with hemolytic activity were present in 10.8% and 5.1% donor samples, respectively. IgG type anti-A and anti-B alone were observed in 9.1% and 3.4% donor samples, respectively while both IgG type anti-A and anti-B were observed in 1.7% donor samples. Gender, age and Rh blood group did not have any significant impact on anti-A and anti-B titer frequencies. To conclude IgM and IgG anti-A and anti-B antibodies exist in significant frequencies and titers among group O donors in Lahore. It is recommended that the transfusion of group O blood to non-O recipients should be done after evaluating the titer of anti-A and anti-B hemolysins.

It is a common assumption that group O individuals are 'universal donors of RBCs' because the RBCs of such persons lack ABO antigens which are the sites of attack for the naturally occurring ABO antibodies found in an individual. In case of emergency situations when the specific ABO blood group is not available, there is a common practice to transfuse blood group O to non-group O individuals. However, there are naturally occurring anti-A and anti-B antibodies in the plasma of

blood group O donors (Oyedeji *et al*., 2015). For its use in emergency situations, several attempts have been made to enzymatically convert blood group A and B to blood group O. But these enzymatically converted blood group RBCs to blood group O RBCs also caused agglutination reactions in some recipients and these reactions were more frequent among previously infected recipients (Rahfeld and Withers, 2020). ABO compatibility is also important for increments in platelet transfusion. Previous studies on minor ABO-incompatible platelets transfusions in allogenic transplants have reported increased morbidity, acute hemolytic transfusion reactions, and veno-occlusive disease (Khampanon *et al*., 2012).

According to a study, blood group O is the frequently occurring blood group in Pakistan (Ali *et al*., 2005). Recent studies on evaluation of anti-A and anti-B titers demonstrated that the plasma of blood group O donors contains both IgM and IgG anti-A and anti-B, and when

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transfused to nonidentical group O mothers, it often results in HDFN. Various studies from different regions of the world reported the prevalence of IgG and IgM type anti-A and anti-B hemolysins among their donor populations. A study from Nigeria reported a prevalence of 55.4% of these hemolysins (Kagu *et al.*, 2010). According to a study in Thailand, the titers of IgM anti-A and anti-B among blood group O donors were higher than that of observed in Japanese group O donors. They found only a significant relation between age of donor and IgM anti-B titer (Khampanon *et al.*, 2012).

Titer of both IgM and IgG anti-A and anti-B antibodies among blood group O donors have been reported by many countries. Recently, a cross-sectional study involving 560 blood group O donors in India further reported the prevalence of IgM and IgG anti-A and anti-B antibodies among these donors (Kannan *et al.*, 2020). There was no other study reporting anti-A and anti-B titers in this region of the world. Due to the life-threatening effects of blood group O transfusion to nonidentical group O individuals, it was necessary to demonstrate the titer of these antibodies in group O donor population.

Materials and methods

A total of 350 blood samples were collected from healthy group O donors (320 males and 30 females) between 18-58 years of age, after screening of blood for the common pathogens. About 4ml venous blood was collected in blood clotting tubes from each blood donor. In house prepared, 5% suspension of A_1 cells, B cells and O cells were used. The samples were centrifuged and the serum was separated in labelled Eppendorf tubes and stored at -18-20˚C until the samples were analyzed for antibodies titer. All samples were analyzed within 24 h of separation.

This cross-sectional observational study was carried out at the Department of Hematology and Transfusion Medicine, The Children's Hospital and Institute of Child Health, Lahore from October 2019 to February 2020. To test for hemolysins in the samples, 100 µl of donor serum was placed in three test tubes. To each test tube 100 µl of 5% suspension of A_1 cells, B cells and O cells was added. O cells served as negative control. The tubes were incubated for 1 h at 37˚C and analyzed for presence of hemolysis against a light source. Grading for hemolysins was done as follows: complete hemolysis = 3 +, partial i.e., > 50% but not complete hemolysis = 2 +, trace hemolysis = 1 + and 0/ negative = no signs of visible hemolysis. Samples showing hemolysis were further evaluated for titer of IgG anti-A and anti-B hemolysins.

Antibody titration for IgM and IgG anti-A and anti-B was performed according to AABB (American Association of Blood Bank) Technical Manual, nineteenth edition (Fung *et al.*, 2017). A volume of 100 µl of each donor serum was double diluted serially in 0.9% saline up to 256. For each sample, the dilutions were prepared two times for determining IgM anti-A and anti-B titers, separately. For preparing dilutions, 10 tubes were labelled according to serial dilutions: 1, 2, 4, 8, 16, 32, 64, 128, and 256, for measuring IgM anti-A titer. The first tube was undiluted serum, tube 2 contained ½ dilution, tube 4 contained ¼ dilution, and so on. To each tube, 100 µl of 0.9% saline was added. Then plasma volume of 100 µl was added to both tubes 1 and 2. By using a clean pipette, the ½ dilution was mixed several times and 100 µl of dilution was transferred to tube 4. The same process was continued for all dilutions (256). Finally, 100 µl of dilution was removed from tube 10. Then, 100 µl of 5% suspension of A_1 cells was added to each tube. To measure the titer of anti-B, a new set of 10 tubes were labelled according to serial dilutions and the process was repeated as mentioned above. Only, 100 µl of 5% B-cells suspension was added to each tube for IgM anti-B. Also, added 100 µl of each donor serum to 100 µl of O cells suspension in a test tube as a negative control. The tubes were then centrifuged at 3400 rpm for 15 seconds. After centrifugation, the tubes were examined microscopically for hemolysis, and the titers were recorded. Titers were recorded as the dilution showing the weakest hemolysis microscopically.

For evaluating IgG anti-A and anti-B hemolysins titers, dilutions were prepared as mentioned above. After adding 5% suspension of A_1 cells and B cells, the tubes were incubated for 1 h at 37˚C and the titers were recorded microscopically. Titers of 64 or greater were considered as high titers. Statistical analysis and calculations were done using the Microsoft Excel 2016 and IBM SPSS statistics 25.0 Software. Graphs and tables were used to display the data. The level of significance for all statistical tests was 5%.

Results

Three hundred and fifty blood samples were collected from healthy and voluntary blood group O donors (325 Rh-D positive and 25 Rh-D negative) with median age 27 years ± 6.4 SD. The sera of the donors were examined to measure the titer levels of IgM and IgG anti-A and anti-B antibodies. Hemolysis was observed in 14.5% (51) donor samples (Fig. 1). The total prevalence of IgG anti-A and anti-B hemolysins was 15.9%. IgG anti-A and anti-B with hemolytic activity were found in 10.8% and 5.1% donor samples, respectively. IgG type anti-A and anti-B alone were observed in 9.1% and 3.4% donor samples, respectively while both IgG anti-A and anti-B were observed in 1.7% donor samples.

Titer values for IgM anti-A and anti-B ranged from 4 to 256 and 2 to 256, respectively as given in Table I. Overall, more than 50% of donors' sera had high titer values (greater than 64) of IgM type anti-A and anti-B. IgM anti-A and anti-B titer levels greater than 64 were observed in 65.6% and 54.9% of donor samples, respectively. The most frequently observed IgM anti-A and anti-B titer was 64 in 29.4% (n = 103) and 32 in 24.9% (n = 87) donor samples, respectively. Lower titer values (less than 64) were more common in anti-B than in anti-A.
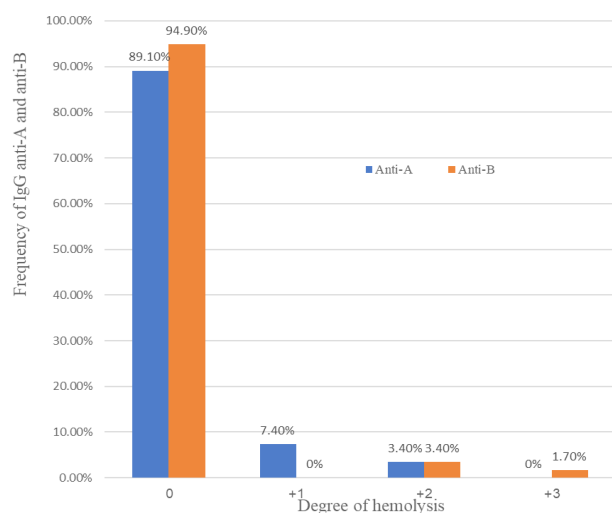

Fig. 1. Frequency of IgG anti-A, anti-B and degree of hemolysis.

Table I. Frequency of IgM and IgG anti-A and anti-B titer and IgQ anti-A and anti-B titer.

Titer values	IgM Anti-A	IgM Anti-B	IgG Anti-A	IgG Anti-B
2	0 (0%)	5 (1.4%)	0 (0%)	0 (0%)
4	9 (2.6%)	4 (1.1%)	4 (7.8%)	0 (0%)
8	7 (2%)	13 (3.7%)	13 (25. 5%)	0 (0%)
16	28 (8%)	49 (14%)	14 (27. 5%)	9 (33.3%)
32	79 (22.6%)	87 (24.9%)	4 (7.8%)	0 (0%)
64	103 (29.4%)	66 (18.9%)	4 (7.8%)	10 (37.3%)
128	88 (25.1%)	69 (19.7%)	12 (23. 6%)	8 (29.7 %)
256	36 (10.3%)	57 (16.3%)	0 (0%)	0 (0%)
Total	350 (100%)	350 (100%)	51 (0%)	27(0%)

Titer values for IgG anti-A and anti-B hemolysins observed in 51 donors' sera ranged from 4 to 16 and 16 to 128, respectively (Table I). The most frequently observed IgG anti-A and anti-B titers were 16 and 64, respectively. Lower titer levels (less than 64) were observed most commonly in anti-A hemolysins as compared to that of anti-B hemolysins.

The mean and median values for both IgM and IgG anti-A titers were greater than that of IgM and IgG types anti-B (Table II).

Table II. Mean, median and SD values for IgM and IgG anti-A and anti-B titer values.

| | IgM | | IgG | |
	Titer anti-A	Titer anti-B	Titer anti-A	Titer anti-B
Mean	86.73	89.56	44.39	69.33
SD	64	64	4.92	4.65
Median	69.6	83.4	16	64
Minimum	4	2	4	16
Maximum	256	256	128	128

Discussion

The transfusion of blood group O to nongroup O recipients has been continued since 2nd World War, however; the presence of IgM and IgG anti-A and anti-B antibodies in blood group O donors sometimes lead to RBCs destruction and acute hemolysis in case of transfusion of single-donor platelet concentrates to nongroup O recipients. The level and type of these antibodies among blood group O persons varies according to ethnicity, environmental factors and geographical regions (de Franca *et al.*, 2011). Data has suggested that both IgM and IgG antibodies can possess hemolytic properties and theoretically titers of IgM antibodies are significant for transfusion and IgG titers are taken into account for transplantation (Denomme and Anani, 2020).

The prevalence of high titer IgM anti-A and anti-B observed in this study are higher than similar studies. IgM titer level > 64 appeared to be 65.6% and 54.9% for anti-A and anti-B in comparison to 38% and 31% (Sood *et al.* 2016) and 36% and 32% (Kannan *et al.*, 2020). Another study by (de Franca *et al.*, 2011) demonstrated the predominance of low titers of IgM anti-A and anti-B antibodies among their study population. The higher titer levels observed could be attributed to ethnic, environmental and lifestyle differences. Studies have shown a decrease in anti-A and anti-B titers in Japanese population over a period of fifteen years. This can be attributed to lifestyle changes of donors. As compared to other Asian populations, the Japanese people consume more processed foods similar to that of North Americans (Denomme and Anani, 2020).

The overall prevalence of IgG anti-A and anti-B hemolysins among blood group O donors in this study was 15.9% which is close to the prevalence of 18.3% reported by (Ek *et al.*, 2013), but is lower than that reported by (Oyedeji *et al.*, 2015) that found a prevalence of 30.3% in Nigeria in 2014 and Kagu *et al.* (2010) that found a prevalence of 55.4% in Nigeria in 2011.

IgG anti-A hemolysins were found to have a higher prevalence than IgG anti-B hemolysins in this study in 10.8% and 5.1% of 350 blood donor samples, respectively. These results are comparable to those obtained by a study in India in which the prevalence of high titer IgG anti-A and anti-B hemolysins came out to be 9.6% and 5.5%, respectively (Kannan *et al.*, 2020). The mean value for IgG anti-B titer was 69.33±4.65 as compared to 44.39±4.92 for IgG anti-A. These findings are not consistent with that of (Olawumi and Olatunji, 2001; Worlledge *et al.*, 1974; Okafor and Enebe, 1985) who all found anti-A hemolysins occurring more frequently than anti-B hemolysins.

The differences in antibody titers observed can also be explained in terms of variations in the sample size, detection procedure, incubation time and concentration of cell suspensions within this study and various other studies. We used 5% RBCs suspension for A_1, B and O cells while some studies used 0.8% and 3% RBCs suspension for testing. Other variations include classifying the titer of hemolysins on the basis of degree of hemolysins (Bastos *et al.*, 2020). Moreover, the gold standard method for titer determination is flow cytometry, especially in the case of ABO-incompatible transplant recipients (Warner and Nester, 2006). Another study that compared different titration methods in the laboratories demonstrated that each method for titer determination could show variable results depending on the type of ABO blood group (Kang *et al.*, 2014). A study analyzing the long-term protection of anti-HBs among vaccinated individuals found higher antibody titers among individuals vaccinated at 12 years of age demonstrating that seroconversion gives better yield among individuals vaccinated at this age (Mastrodomenico *et al.*, 2021). However, in this study no significant effect of gender, Rh blood group and age was observed on the frequency of IgM and IgG anti-A and anti-B antibodies.

Conclusion

High titer IgM antibodies and IgG hemolysins do exist in significant levels in our blood group O donor population. There is a need for screening of these hemolysins when issuing blood to nongroup O recipients. Moreover, the use of blood group O to nongroup O recipients should be avoided unless inevitable. Future studies should focus on the clinical significance of these hemolysins among nongroup O recipients of RBCs, platelet concentrates or transplantation.

Statement of conflict of interest

The authors have declared no conflict of interest.

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