RESEARCH ARTICLE

Prevalence of anti A and anti B titer among blood group O donors

Alia Maisarah Mohd Azli, Mazura Bahari*

Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA Cawangan Selangor Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia.

***Corresponding Author**

Mazura Bahari
Email:
mazurabahari@uitm.edu.my

Abstract:

Blood group O is well known as universal donors. However, some of the antibodies from group O, which are anti A and anti B may able to cause hemolysis of recipient's red blood cells if it is presented in sufficient high titer. Therefore, this pilot study was conducted to determine the prevalence of anti A and anti B titer among the blood group O donors. This study was carried out on 30 test tubes of blood, originated from leftover samples. All the samples from blood group O donors had been chosen randomly. Anti A and anti B antibody titration using the conventional tube technique had been done. Pearson Chi-square and Fisher's Exact test was used to compare the association between titration value and types of antibodies. This study showed that the prevalence of anti A and anti B titer among blood group O donors is 90.0% at the titer lower than 128. The critical value of 'high-titer' is also at the titer lower than 128. The result showed that there is no significance association between titration value and types of antibodies ($p > 0.05$). In conclusion, the blood group O donors can be accepted as the universal donors and can be released as safe O when the titer is lower than 128.

Keywords: Blood, Group O, Anti A, Anti B, Transfusion

1. INTRODUCTION

In Malaysia, transfusion medicine is popular among individuals (Lim M L et al., 2018.) When an emergency happens, most of the medical team give blood group O to the patient (Thomas and Wee et al., 2010). This is because of blood group O known as the universal donor as blood group O has neither A nor B antigen on the surface of red blood cell that can trigger the antibody in the recipients' red blood cells (Ranadhir M et al, 2014). The discovery of the ABO system from Karl Landsteiner's first experiments marked the beginning of scientific transfusion medicine (Figl & Pelinka, 2004).

Anti A and anti B is a type of immunoglobulin mostly IgM found in blood group system (Branch, 2017). Immunoglobulin is a protein produced by plasma cells and lymphocytes that have a function in the immune system of the body. IgM is the predominant anti A or anti B Abs immunoglobulin (Ig) class produced by group A or B, whereas IgG is the predominant anti A and anti B Abs class in group O serum (Kang, Lim, & Baik, 2014).

Despite its universal donor advantages an adverse effect may occur in certain conditions during blood transfusion of blood group O donors due to anti-A and anti B antibodies. If a high titer of these antibodies present in the plasma of blood group O donor, it may cause hemolysis of red blood cells recipients. (Journal et al., 2016). This is due to hemagglutinins formed when non-blood group O recipient erythrocytes agglutinate with the anti A and B antibodies after plasma of blood group O transfusion. Thus, the purpose of this research is to determine the prevalence titer of anti A and anti B among blood group O donors to prevent transfusion reactions.

2. MATERIALS AND METHODS

A pilot study was done by using 30 leftover samples from blood group O donors that had been chosen randomly. The anti A and anti B antibody titration was measured by using the conventional tube technique had been done.

Standard tube method (serial dilution) has been used to evaluate the agglutinin levels in donors' blood group O. The titration of anti-A and anti-B hemagglutinins are performed by serial dilutions of donor plasma in ethylenediaminetetraacetic acid (EDTA) using saline solution. Based on Figure 1 for the master dilution, 10 test tubes have been placed in a test tube rack and label them

according to the serum dilution. The last tube has been labeled as a control. 0.5 ml of isotonic saline was added to all tubes except the first test tube. 1 ml of antibody-containing serum have been added to test tube 1 only. The contents of test tube 1 has been mixed gently several times. 0.5 ml of the content from test tube 1 was transferred to test tube 2. Mixing and transferring was continuing up to test tube 9. The balance volume taken from tube 9 has been discarded. For the test tube 10 (control tube), anything does not be transferred. For the serial dilution, the required number of test rows was labeled immediately below the master dilution. The same labeling method was used as applied to the master titration earlier. 2 drops of each dilution have been added to the appropriate tubes in the test row. Then, 1 drop of the corresponding red cells (blood group A and B) was added to be tested to each tube in the test row. All tubes were mixed by shaking gently. Inspect each tube to ensure that it has received red cells and the levels were approximately equal. Next, all the test tubes have been centrifuged at 3400 rpm for 15 seconds. Lastly, all the test tubes were read and grade agglutination accordingly.

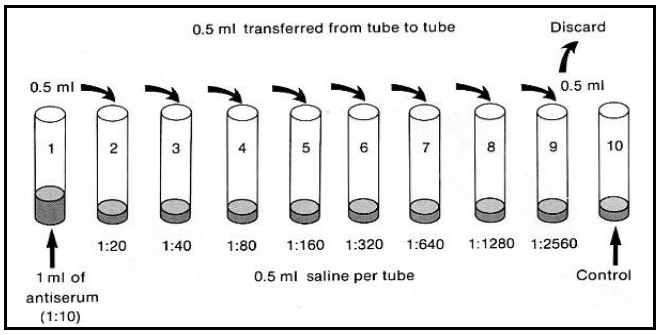

Figure 1: Titration method. The diagram shows the method of serial dilution for titration.

3. RESULT AND DISCUSSION

3.1. Prevalence of anti A and anti B titer among blood group O donors.

Based on Table 1, the prevalence for titer results 1:32 were: anti-A < 32 = 3.3%, ⩾ 32 = 96.7% and anti-B < 32 = 0.0%, ⩾ 32 = 100.0%. Moreover, based on Table 2, the prevalence for titer results 1:64 were: anti-A < 64 = 23.3%, ⩾ 64 = 76.7% and anti-B < 64 = 26.7%, ⩾ 64 = 73.3%. Then, based on Table 3, the prevalence for titer results 1:128 were: anti-A < 128 = 93.3%, ⩾ 128 = 6.7% and anti-B < 128 = 86.7%, ⩾ 128 = 13.3%. For titration 1:32, anti A titers at the titer value < 32 were significantly higher than the anti B titers. At the titer value ⩾ 32, the anti A titers were significantly lower than the anti B titers. In addition, anti A titers were significantly lower han anti B for titration 1:64 at the titer value < 64. But then, at a titer value of ⩾ 64, anti A titers were much greater than anti B titers. At the value of the titer < 128, the anti A titers were significantly higher than the anti B titers and then slightly lower at the value of the titer at ⩾ 128. Anti A titers were considerably greater than anti B for the general consequence that we assume < 128 as the critical value.

Table 1: The prevalence of titer for titration 1:32

			Titration 1:32		Total
			< 32	≥ 32	
Types of antibodies	Anti A	Count	1	29	30
		Expected Count	0.5	29.5	30.0
		% within Types of antibody	3.3 %	96.7 %	100.0 %
	Anti B	Count	0	30	30
		Expected Count	0.5	29.5	30.0
		% within Types of antibody	0.0 %	100.0 %	100.0 %
Total		Count	1	59	60
		Expected Count	1.0	59.0	60.0
		% within Types of antibody	1.7 %	98.3 %	100.0 %

Table 2: The prevalence of titer for titration 1:64

			Titration 1:64		Total
			< 64	≥ 64	
Types of antibody	Anti A	Count	7	23	30
		Expected Count	7.5	22.5	30.0
		% within Types of antibody	23.3 %	76.7 %	100.0 %
	Anti B	Count	8	22	30
		Expected Count	7.5	22.5	30.0
		% within Types of antibody	26.7 %	73.3 %	100.0 %
Total		Count	15	45	60
		Expected Count	15.0	45.0	60.0
		% within Types of antibody	25.0 %	75.0 %	100.0 %

3.2. Critical value

Based on Table 3, the critical value of 'high-titer' is < 128. It is demonstrated by the total percentage within types of antibodies which is 90.0% from 30 blood group O donors. The prevalence for titer results 1:128 were: anti-A < 128 = 93.3%, ⩾ 128 = 6.7% and anti-B < 128 = 86.7%, ⩾ 128 = 13.3%.

Table 3: The prevalence of titer for titration 1:128

			Titration 1:128		Total
			< 128	≥ 128	
Types of antibody	Anti A	Count	28	2	30
		Expected Count	27.0	3.0	30.0
		% within Types of antibody	93.3 %	6.7 %	100.0 %
	Anti B	Count	26	4	30
		Expected Count	27.0	3.0	30.0
		% within Types of antibody	86.7 %	13.3 %	100.0 %
Total		Count	54	6	60
		Expected Count	54.0	6.0	60.0
		% within Types of antibody	90.0 %	10.0 %	100.0 %

3.3. Titration value and types of antibodies

Table 4 shows the association between titration value and types of antibodies. There is no significance association between titration value and types of antibodies ($p > 0.05$).

Table 4: The association between titration value and types of antibodies

Types of antibody	Titration < 128 n (%)	Titration ≥ 128 n (%)	p-value*
Anti A	28 (93.3)	2 (6.7)	0.389
Anti B	26 (86.7)	4 (13.3)	

* Fisher's Exact Test

DISCCUSION

Blood group O donors are often used as universal donors, but group O plasma transfusion sometimes leads to red blood cells being destroyed. In blood group O donors, the destruction of red blood cells in recipients seems to be influenced by anti A and anti B titer. Hemolytic reactions were discovered after the following transfusion of blood products owing to an adverse effect contributed by dangerous universal donors (Lozano & Cid, 2003). This study showed that the prevalence of anti A and anti B titer among blood group O donors is 90.0% at the titer lower than 128. Table 5 summarizes the outcomes of titration frequency, prevalence of anti A and anti B. This study showed that the prevalence of anti A and anti B titer among blood group O donors for titration < 32 anti A is 1 and anti B is 0 while for titration ⩾ 32 anti A is 29 and anti B is 30. There is only 1% difference between the prevalence of anti A and anti B for titration 1:32. Moreover, the prevalence for titration < 64 anti A is 7 and anti B is 8 while for titration ⩾ 64 anti A is 23 and anti B is 22. The difference between the prevalence of anti A and anti B for titration 1:62 also only 1%. The prevalence for titration < 128 anti A is 28 and anti B is 26 while for titration ⩾ 128 anti A is 2 and anti B is 4. The difference between the prevalence of anti A and anti B for titration 1:128 is 2%. Hence, it is suggested that there is only 1-2% difference between the prevalence of anti A and anti B titer among blood group O donors.

Table 5: The differences between the prevalence of anti A and anti B

Titration Value	Frequency		Difference Between Prevalence of Anti A & Anti B (%)
	Anti A	Anti B	
< 32	1	0	1
≥ 32	29	30	
< 64	7	8	1
≥ 64	23	22	
< 128	28	26	2
≥ 128	2	4	

The critical value of 'high-titer' for anti A and anti B based on this method was < 128. It is showed by 90.0% of the total percentage within types of antibodies among blood group O donors at the titration < 128. According to a study conducted in the United States, 28% of the samples were labeled as high titer using 64 or higher than the cut off while the average IgM titer was 32 in gel of 100 O apheresis donors (Josephson et al., 2004). The high titer or critical value for IgM in tube or gel is 64-100 respectively (Pietersz et al., 2005. According to Sadani et al. (2006), specifically at the blood collection facilities in the UK, they used automation to screen donor units. By using the manual tube titer method, the products containing anti A or anti B antibodies in a titer above 100 were commonly accepted as equal to a titer of 128. However, by using this European

strategy of a single dilution cut-off, it is still not fully proven and has some residual risk. So, based on all the study above, it is correlated with the critical value in our study.

Moreover, the prevalence of dangerous universal donors in this study were 10.0% of the total percentage within types of antibodies. It is due to the 6.7% anti A and 13.3% anti B. There are a few studies have investigated in Brazil about the frequencies of dangerous universal donors in blood banks. In Botucatu, São Paulo, 12.8% of blood group O donors were classified as dangerous (Gambero et al., 2005). It is due to the 58.4% anti A IgM antibodies and 14.2% anti B IgM antibodies. Additionally, in Guarapuava, the study found that the frequency of dangerous universal donors was 7.3%. 44.4% of them due to anti A IgM antibodies and 35.6% due to anti B IgM antibodies (Cosechen et al., 2009). The differentiation could be due to the methods and the big sample size used between the present study and the prior study.

To define dangerous universal donors with the titers is difficult. This is because of disagreements regarding titration techniques and the delineation of critical limits. By using microplate titration technique, many studies use a cut-off point of 100 and classify the donor as a dangerous donor (Journal et al., 2016). However, based on the AABB Technical Manual about the definition of titer, the standardization in the reading of agglutination intensities using this technique is more complex compared to the tube technique. The titer that produces a macroscopic agglutination of one cross (1+) stated as the highest dilution (Roback et al.,2011).

This study showed no significance association between titration value and types of antibodies (p>0.05). Unfortunately, we do not have information on our donor population's sex, age, and ethnic background. Therefore, the reasons that there is no significance association between titration value and types of antibodies could not be investigated. The study needs to vary in approximately every detail for further complexity. It includes the use of plasma or serum donor and/or patient, the medium used or dilution, time of incubation and speed of centrifugation, use of polyclonal vs. monoclonal secondary step antibody for indirect agglutination.

The genetic background and socioeconomic status may explain the results (Pérez et al., 2010). Furthermore, the level of antibodies will rely on the ethnic background and the environment (Sood R et al., 2018**).** Based on a study in the blood bank of Belo Horizonte, there was no significant association between the antibody class and being classified as a dangerous universal donor. They also did not find any significant association between the specificity of hem agglutinins (Journal et al., 2016).

But a straightforward method is used in this current study and donor data is not complete. In Malaysia, the precise critical value for blood group O donors of' 'high titer' is not yet approved. In addition, we need to develop some methods to improve the safety of blood group O products with the abundance of blood group O donors. In European, they have defined a safe level of isohemagglutinins for their donors and a cut-off determination to label products when the titer is high and thereby restrict its use (Josephson, et al.2010). However, the greatest challenge is to convince the authorities to establish a safe level of ABO antibodies in blood group O donors at a national level.

4. CONCLUSION

The prevalence of anti A and anti B titer among blood group O donors is 90% at the titer lower than 128. There is no significance association between titration value and types of antibodies. In conclusion, the blood group O donors can be accepted as the universal donors and can be released as safe O when the titer is < 128.

ACKNOWLEDGEMENTS

The author would like to thank to all the staff in Blood Banking, from the Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia, that participated in this study.

REFERENCES

Branch, D. R. (2017). Anti-A and anti-B: what are they and where do they come from? *Transfusion*, (July 2015). https://doi.org/10.1111/trf.13087

Cosechen, V. de S., Pittner, E., Khalil, N. M., Horst, S., & Monteiro, M. C. (2009). *Frequencies of Anti-A and Anti-B agglutinins in blood donors of the group O in the Guarapuaza hemonuclear blood donors (PR)*.

D Thomas and M Wee, Joint Chairmen, P Clyburn, I Walker, K Brohi, P Collins, … L Shewry. (2010). *Blood transfusion and the anaesthetist : management of massive haemorrhage*. 1153–1161. https://doi.org/10.1111/j.1365-2044.2010.06538.x

Figl, M., & Pelinka, L. E. (2004). *Karl Landsteiner , the discoverer of blood groups*. *63*, 251–254. https://doi.org/10.1016/j.resuscitation.2004.09.001

Gambero, S., Secco, V. N. D. P., Ferreira, R. R., Deffune, E., & Machado, P. E. A. (2005). Freqüência de hemolisinas anti-A e anti-B em doadores de sangue do Hemocentro de Botucatu. *Revista Brasileira de Hematologia e Hemoterapia*, *26*(1). https://doi.org/10.1590/s1516-84842004000100006

Josephson, C. D., Mullis, N. C., Van Demark, C., & Hillyer, C. D. (2014), "Significant numbers of apheresis-derived group O platelet units have "high-titer" anti-A/A,B: Implications for

transfusion policy. *Transfusion*," 44(6), 805–808.

Josephson, Cassandra D., Castillejo, M. I., Grima, K., & Hillyer, C. D. (2010). ABO-mismatched platelet transfusions: Strategies to mitigate patient exposure to naturally occurring hemolytic antibodies. *Transfusion and Apheresis Science*, *42*(1), 83–88. https://doi.org/10.1016/j.transci.2009.10.013

Journal, B., Martins, M., Oliveira, L. De, Cayres, L., Mello, L., Silva, R., Dusse, S. (2016), "Revista Brasileira de Hematologia e Hemoterapia Original article Dangerous universal donors: the reality of the Hemocentro in Belo Horizonte, Minas Gerais. Revista Brasileira de *Hematologia e Hemoterapia*," 38(3), 193–198.

Kang, S. J., Lim, Y. A., & Baik, S. Y. (2014). *Comparison of ABO Antibody Titers on the Basis of the Antibody Detection Method Used*. 300–306.

Lim Mei Ling, Thock Sheng Hui, Andrew K. G. Tan, & Gwee Sai Ling. (2018). *Published online: 27 April 2018 To cite this article: Lim, M. L., S. H. Thock, A. K. G. Tan and S. L. Gwee. 2018. Determinants of blood donation status in Malaysia: Profiling the non-donors, occasional donors and regular donors*. *36*(1), 43–62.

Lozano, M., & Cid, J. (2003). The clinical implications of platelet transfusions associated with ABO or Rh(D) incompatibility. *Transfusion Medicine Reviews*, *17*(1), 57–68. https://doi.org/10.1053/tmrv.2003.50003

Pérez, N., Iannicelli, J. C., Girard-Bosch, C., González, S., Varea, A., Disalvo, L., … Cravero, R. (2010). Effect of probiotic supplementation on immunoglobulins, isoagglutinins and antibody response in children of low socio-economic status. *European Journal of Nutrition*, *49*(3), 173–179. https://doi.org/10.1007/s00394-009-0063-5.

Pietersz, R. N. I., Engelfriet, C. P., Reesink, H. W., Davis, K. G., Lown, J., Thomson, A., … Blumberg, N. (2005). Transfusion of apheresis platelets and ABO groups. *Vox Sanguinis*, *88*(3), 207–221. https://doi.org/10.1111/j.1423-0410.2005.00615.x

Ranadhir Mitra, Nitasha Mishra, & Girija Prasad Rath. (2014). *Blood groups systems*. *58*(5), 524–528. https://doi.org/10.4103/0019-5049.144645

Roback, J. D., Grossman, B. J., Harris, T., & Hillyer, C. D. (2011). *AABB Technical Manual, 17th Edition*. 17.

Sadani, D. T., Urbaniak, S. J., Bruce, M., & Tighe, J. E. (2006). Repeat ABO-incompatible platelet transfusions leading to haemolytic transfusion reaction. *Transfusion Medicine*, *16*(5), 375–379. https://doi.org/10.1111/j.1365-3148.2006.00684.x

Sood R, Neelima, Kumar D, Kumar T, Kumar V, Rani S, & Kumar S. (2018). Antibody Titers Study in Group O Blood Donors: Tube and Column Agglutination Techniques. *Journal of Thrombosis and Circulation: Open Access*, *02*(01), 1–6. https://doi.org/10.4172/2572-9462.1000104

