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Research paper

Detection of anti-A and anti-B haemagglutinin titre in blood group O donor plasma and its correlation to donor age and gender

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Key words: acute haemolytic transfusion reactions, universal donor, haemagglutinin titre, titration techniques

Abstract

Although blood group O donors are considered as universal donors, their plasma contains both types of antibodies; anti-A and anti-B. These antibodies can cause acute haemolytic transfusion reactions (HTR) due to passive transfer of antibodies. These antibodies can be detected using titration techniques. Titers 1:64 or higher is considered as the high tittered antibody levels for IgM type anti-A and anti-B and titers 1:32 or lower is considered as low tittered antibody levels. This cross-sectional analytical study was aimed to determine the anti-A and anti-B haemagglutinin titre in blood group O donor plasma and its correlation to donor age and gender. The study sample consist of a total of 132 blood group O donor samples received to the blood bank, Teaching Hospital Karapitiya (THK), over a period of one month from March to April 2022. Samples were analyzed using standard tube technique. The study sample consisted of 44.6% females and 55.3% males, and their ages ranged from 18 to 60 years. In this sample there were 45.5% donors with high antibody titres and it was observed that the donor frequencies for both anti-A and anti-B titres were higher in females than in males. The highest titres (1:128) were observed among female donors aged 29-38 years. Furthermore, the results of this study show a significant correlation between donor age and high titers among young donors, compared to older donors. Among both female and male donors, the highest antibody titres were found in age groups 18-28 and 28-38. Therefore, the risk of being a dangerous donor is higher among younger donors (18-38) than the older donors (48-55). Findings of this study confirm the necessity to perform prior titrations during transfusion of blood group O donor products, especially for products containing high volume of plasma. Low tittered O donor blood products can be used for safe transfusions during out of group transfusions, preventing transfusion reactions.

Introduction

One of the major donor considerations in blood transfusion is to confirm whether the donation is safe for both donor and patient. Under the recipient consideration proper identification of the unit, appropriateness of blood as the best treatment modality, unit administration and recipient evaluation are considered[6]. After collection, the blood should be tested for safety such as infectious disease testing, compatibility testing and for other necessary modifications.

At present 33 blood group systems representing over 300 antigens are listed by the International Society of Blood Transfusion. Among these 33 blood group systems ABO remains the most important in transfusion and transplantation since any person above the age of 6 months has clinically

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significant anti-A and anti-B antibodies in their serum[13].

Anti-A and anti-B are potent immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. They bind to A and B antigens on the outer membrane of erythrocytes and may activate the complement cascade resulting in acute intravascular haemolysis[5].

If there is an incompatibility between donor blood cells and recipient plasma, it is called major incompatibility. If there is an incompatibility between donor plasma and recipient blood cells, it is called minor incompatibility. Though minor incompatibility is rare than major incompatibility both can be fatal[2].

Though the blood group O donor is considered as the universal donor, there can be acute hemolytic transfusion reactions (AHTR) due to the presence of high titres of anti-A and anti-B haemagglutinins in some donor plasma[12]. AHTR are the reactions which occur within 24 hours of transfusion[3]. AHTR are caused due to passive transfer of antibodies to group A or group B cells. This haemolysis is more common in ABO incompatible platelet transfusions. In ABO mismatched platelet transfusions, there can be rare but severe complications of haemolysis. This can be seen especially in single donor platelets[7].

During an incompatible blood transfusion if the volume is more than 50.0 mL there can be severe AHTR[4]. There are methods to define the safety level of isohaemagglutinins in order to transfuse mismatched blood products[8]. Thus prior titration of anti-A and anti-B haemagglutinins is recommended to find out the high titre group O blood, to prevent AHTR. Although many studies have been conducted in other countries about high titre group, not many have been conducted in Sri Lanka. The objective of this study was to detect the anti-A and anti-B haemagglutinin titres in blood group O donor plasma and find its correlation to donor age and gender.

Methodology

This research is a cross-sectional analytical study conducted using 132 blood group O donor blood samples collected at the blood bank, THK. The age range of the study sample was 18 to 55 years, including 73 males and 59 females. The samples collected within a period of one month from March to April in 2022 were used for the study. The ethical clearance was obtained from, the Ethical Review Committee, Faculty of Allied Health Sciences, University of Ruhuna. Permission to use retained donor ethylene diamine tetra acetic acid (EDTA) blood samples and also the permission to collect relevant donor details from donor declaration form was obtained from the Director of National Blood Center and from the Director THK. The laboratory procedures were carried out in student laboratory of Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Ruhuna.

For this study, sample collection was conducted using convenience sampling method. The retained O donor blood samples were collected and samples were stored at 2-8°C until the analysis, keeping the maximum time period only up to 7 days. Relevant donor data (donor age and gender) were collected from donor declaration form. Samples were analyzed using standard tube technique.

Analysis using standard tube technique

Group O donor plasma contains anti-A and anti-B. After addition of 5% cell suspensions of A1 cells and B cells separately, anti-A reacts with A1 cells and anti-B reacts with B cells causing agglutination. Agglutination strength depends on the concentration of anti-A and anti-B. Anti-A and anti-B concentration was changed by making a dilution series of donor plasma in a descending order.

Anti-A and anti-B both contain IgG and IgM types which give agglutination, although major contribution is from IgM. Therefore, the inverse of haemagglutinin titre mainly represents the level of IgM type.

In this experiment three sets of two-fold dilution series were performed using three rows of 11 test tubes per one donor labeled as anti-A titre, anti-B titre and control. Dilution series was prepared as 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024.

Anti-A titre was detected using 5% suspension of A1 cells, anti-B titre was detected using B cells. A 5% suspension of O cells was used as the control. After incubation of each set of tubes under room temperature for 15 minutes, they were centrifuged at 1000 rpm for 1 minute. An agglutination reading was performed visually for each tube.

Data analysis

The correlation between haemagglutinin titre and donor age and gender was demonstrated using correlation coefficient (r) and p values ≤0.05 were considered as significant. All the data were analyzed using statistical package for the social sciences (SPSS version 25).

Results

Out of the 132 samples evaluated, 59 (44.6%) were females. Regarding the age, 43 (32.6%) donors were between 18-28 years, 44 (34.1%) were between 29-38 years, 30 (22%) were between 39-48 years and 15 (11.4%) were between 49-55 years.

In the study population there were 45.5% of donors with high titres (1:64) and 54.5% of donors with low titres (1:32). It was observed that the donor frequencies for both anti-A and anti-B high titres were higher in females than in males. The highest titres (1:128) were observed among female donors aged 29-38 years. The distribution of anti-A and anti-B titre levels with donor age and gender in the study population are shown in Figures 1-4.

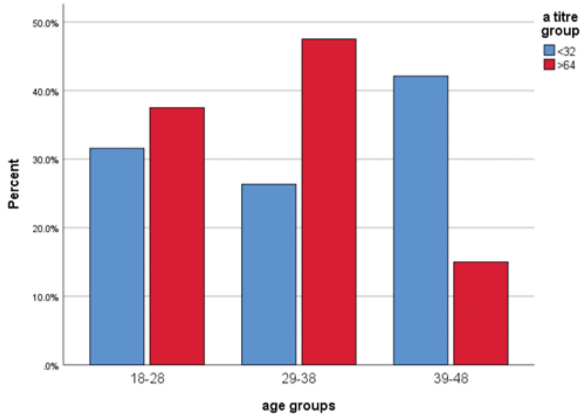

Figure 1. Frequency of anti-A titre with donor age groups in females.

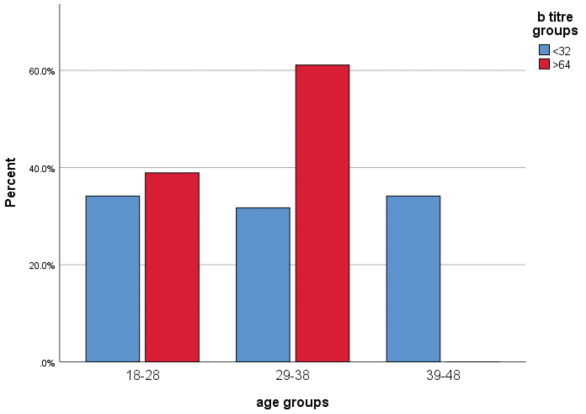

Figure 2. Frequencies of anti-A titre with donor age group in males.

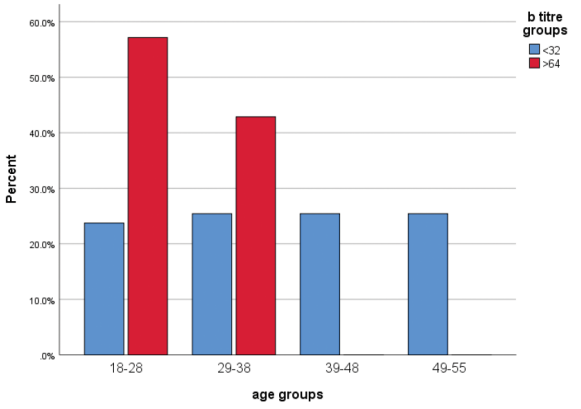

Figure 3. Frequencies of anti-A titre with donor age group in males.

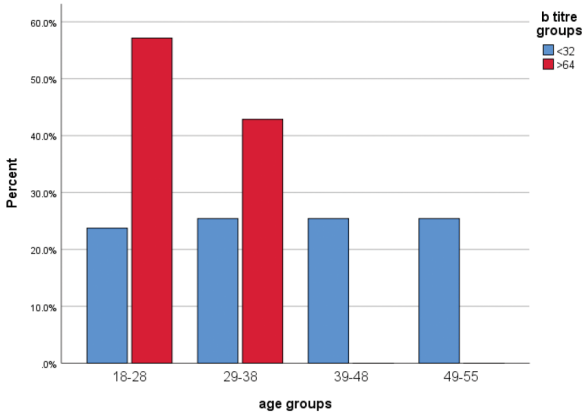

Figure 4. Frequencies of anti-B titre with donor age group in males.

Table1. Mean anti-A titre and anti-B titre in donor age groups

Age group (years)	Male/female	Mean anti-A titre	Mean anti-B titre
18-28	Female Male	1:64 1:64	1:32 1:32
29-38	Female Male	1:128 1:32	1:64 1:32
39-48	Female Male	1:32 1:16	1:16 1:08
49-55	Female Male	- 1:08	- 1:08

It should be noted that there were no males having high titres of anti-A and anti-B in the older age groups (39-48 and 49-55). Also, there were no females with high titres of anti-B in the age group of 39-48 years. The mean anti-A and anti-B titres in age groups among males and females are shown in Table 1.

When considering the correlation between age group and gender, it should be noted that there was a statistically significant negative correlation ($p<0.05$) between donor age group and anti-A titre (-0.733) as well as with anti-B titre (-0.756) among male donors as shown in Figure 5 and 6.

The correlation between donor age group and anti-A titre (-0.235) as well as with anti-B titre (-0.331) among female donors were not statistically significant as in males.

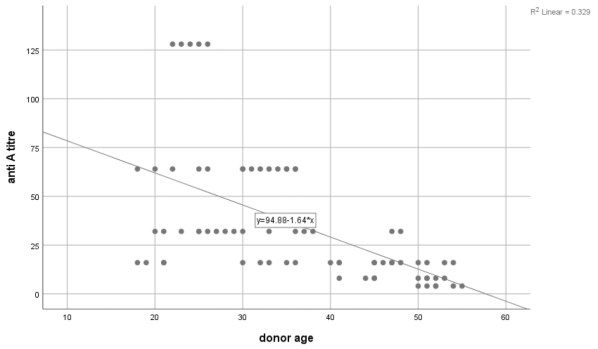

Figure 5. Correlation between anti-A titre and donor age in males.

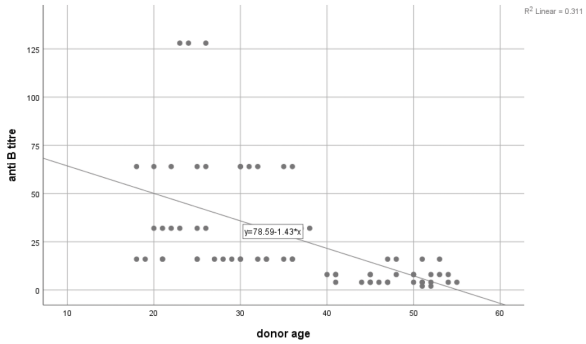

Figure 6. Correlation between anti-B titre and donor age in males.

Discussion

Worldwide many cases have been reported on haemolytic transfusion reactions due to the passive transfer of antibodies. It is known that higher the plasma content in blood products, there is a higher risk of acute haemolytic transfusion reactions[11]. This study was conducted to detect the anti-A and anti-B haemagglutinin titre in blood group O donor plasma and find its correlation to donor age and gender in Sri Lankan population. For this study, the cutoff values which were considered to clarify the donors as high tittered was 1:64 for both anti-A and anti-B titres.

According to this study the females in age group 29-38 years had the highest titres (1:128) among all age groups. Considering the age group, the

results of this study show a significant correlation between donor age and high titers among young donors, compared to older donors. Therefore, the risk of being a dangerous donor is more among young donors (18-28) than the older donors (48-55). This finding is correlated to the study from India, where it showed the men aged >40 years have very low titres and more reduction was observed beyond age 50[9].

Also it is known that the anti-A haemagglutinin titer is higher than anti-B haemagglutinin titer in blood group O donor plasma[1]. Further, it is known that the strength of the haemagglutinin titre of anti-A is higher than anti-B[1]. However, the results of this study show that there were 18% of group O donors who had higher anti-B titre than anti-A titre.

Another study conducted reported that 50% high titres observed when they considered 1:150 as the titer cut off, 33% when they considered 1:200 and 25% when they considered 1:250 as the cut off[14]. Therefore, an exact cut off cannot be given universally as it varies among populations.

For this study standard tube technique with double dilution method such as 1:2, 1:4 and 1:8 was used. It should be noted that the titre findings would depend on the method of dilution. Furthermore, some studies have used gel cards for titres while some used increasing dilution series or doubling dilutions[9]. In some studies all the samples were analyzed with a single dilution, using a single cut off to clarify the samples as positive or negative for high titres[14].

Documentation on prior titration of anti-A and anti-B helps to predict the risk of haemolysis and may help to avoid using such O group donors as universal donors[9].

Many studies have shown a statistically significant association between donor gender and critical titres[9]. As observed in this study, many studies have shown that the female gender has a greater association with high antibody titres[9]. There is evidence that the number of female donors with higher titres had grown in recent years as pregnancy is a factor that contributes to higher anti-A and anti-B haemagglutinins[10].

When considering the females a correlation between donor age and titre could not be proved to be statistically significant because of the skewed data distribution among age groups as there were no female donors in age group 49-55 years.

There were some results which didn't show the acceptable results due to many influencing factors such as probiotic use, subclinical infections and geographic variations[9].

Conclusions

Findings of this study confirm the necessity to perform prior titrations during transfusion of blood group O donor products especially, products containing high volume of plasma. If this titration technique could be established in routine blood bank practice, it would contribute a better uninterrupted patient management for patients who benefit from blood components from O blood group donors. Such practice would be helpful to exclude O donors who have high titres of antibodies, thus, low titred O donor blood products can be used for safe transfusions during out of group transfusions, preventing transfusion reactions.

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Authors declare no conflicts of interest.

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