Original article

Determination of ABO antibody titre and haemolysin test of group O whole blood used for exchanged transfusion in a teaching hospital

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Abstract

Background: Group O blood donor is more readily available and is frequently used as a universal red cell donor and emergency O whole blood (EM O) for exchange transfusion in neonates. Sera from group O donors contains two separable haemolysin antibodies, anti-A and anti-B, and a cross-reacting antibody called anti-A, B (mostly IgG). The presence of haemolysins in the donors may lead to haemolysis in the recipients. ***Objectives***: This study aims to determine the prevalence of anti-A and anti B haemolysins and titer among group O donors that we screened for emergency group O (EM O) whole blood.***Methodology*:** A cross sectional study was done to determine the prevalence of anti-A and anti B haemolysin and titer among group O blood donors screened for EM O whole blood for exchange transfusion in neonates at a teaching hospital from January to December 2018. Samples of 350 voluntary group O regular donors were selected for ABO antibodies titration and haemolysin test using the conventional tube technique at room temperature. Donors were screened for titer of 1:50 and 1:100 only. ABO antibody titer of ≥1:100 was considered as a high titer and not suitable for exchange transfusion. Titer of ≤1:50 were labeled as EM O whole blood and will be suitable for exchange transfusion. ***Results*:** A total of 350 group O blood donors were screened for the anti A and anti B haemolysin and titer. The majority of blood group O donors were male (n=215, 61%) and were age from 18-30 years old (51%). Malay was the predominant group (83%). About 52.9% of the donors were low titer (≤1:50), and 47.1% were high titer (≥1:100). Low titer was seen predominantly among male donors (61.8%). The prevalence of haemolysins in group O donors was 5.4% (n=19). Anti A and anti B haemolysins were seen in 0.57% and 2.28% of the donors, respectively, while donors having both A and B haemolysins in their sera was 2.57%. ***Conclusion*:** Prevalence of anti-A and anti-B haemolysins were low among group O blood donors. However, a significant percentage of group O donors have high titer of anti-A or anti-B. Therefore, despite the labour intensiveness of haemolysis titration technique and the frequent transfusion of group O blood for exchange transfusion, there is the need to routinely screen our donors for haemolysins in order to identify those posing the greatest risk to recipients.

Keywords: ABO antibody, anti A, anti B, titer, haemolysis

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Introduction

ABO blood group system is characterized by the presence of natural anti A and/or anti B antibodies in the plasma corresponding to the antigens absent on the membrane of the red blood cell. All group O individuals produce naturally occurring IgM anti-A and anti-B which is a result of stimulation by A- and B-like antigens commonly found in the environment and food[1]. In addition to these natural antibodies, there may be immune antibodies (called haemolysins) in response to different types of immunological stimuli or become sensitized to foreign antigens through pregnancy or transfusion[2-3].

Group O blood is more readily available and is frequently used as a universal red cell concentrate for top-up transfusion or emergency O whole blood for exchange transfusion in neonates with haemolytic disease of fetus and newborn (HDNF). Sera from

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group O donors contains two separable haemolysin antibodies, anti-A and anti-B, and a cross-reacting antibody called anti-A, B (mostly IgG).The presence of haemolysins in the donors may, however, lead to haemolysis in the recipients[2, 4].

ABO antibodies are highly clinically significant, and, because of this, blood cross-matching is done to ensure that blood of the correct type is transfused into recipients to avoid major mismatch or major incompatibility that can cause significant morbidity and mortality[5]. Anti-A and anti-B are naturally occurring antibodies of the IgM class and are present in practically all humans who lack the corresponding antigen. These antibodies will react immediately with a transfused antigen positive cell and in most cases result in an intravascular lysis of the cell[6].

If ABO-compatible blood products were not available, "low titer" group O whole blood or blood components containing a minimum of ABO-incompatible plasma should be used[6]. Transfusion of group O blood with a low titer of anti-A and anti B will minimize the risk for a haaemolytic reaction, particularly for an immunised patient, e.g., post-blood transfusion, vaccination, or pregnancy [7]. The use of O group red cells suspended in A or B plasma for the exchange transfusion in cases of ABO haemolytic disease, decreased the re-exchange risk significantly, compared with O group whole blood[8].

The aim of this study is to determine the prevalence of anti-A and anti B titer and haemolytic activity among group O donors that we screened for emergency group O (EM O) whole blood.

Material and Methods

A cross sectional study was done to determine the prevalence anti-A and anti B titer and haemolytic activity among group O blood donors screened for EM O whole blood for exchange transfusion in neonates at a teaching hospital from January to December 2018. Samples of 350 voluntary group O regular donors were selected for ABO antibodies titration and haemolysin test.

Donors were screened, and titration was done for titer of 1:50 and 1:100 only. ABO antibody titration was done by using the conventional tube technique at room temperature. Diluted sera were incubated in glass tubes at room temperature with a 3% commercial suspension of red blood cells (A1 and B - Diacell, Diamed®). Samples were centrifuged at 3, 400 rpm for 15 seconds. Agglutination was considered positive if the red blood cells remained agglutinated after gentle shaking. ABO antibody titer of ≥1:100 was considered as a high titer and not suitable for exchange transfusion. Titer of ≤1:50 were labeled as low titer whole blood or EM O and will be used for exchange transfusion. There was no consensus on a definitive value for critical titers which will predict in vivo haemolysis. The majority cited the critical high titer haemolysin as cut-off titer of greater than 1:64 for IgM and greater than 1:256 for IgG. Titers of at least 64 and/or 256 were considered critically high based on cited literature[9-11].

Known A and B red cells were used to test the subject's sera for agglutination and subsequent hemolysis of the red cells in the presence of haemolysin and complement in the fresh sera samples. One volume of serum and one volume of 5% red cells suspension of A and B cells were placed into each test tube respectively.The tubes were then incubated at 37°C for 2hrs after which all tubes were then centrifuged at 3400rpm for 15 seconds. The solutions were then examined macroscopically in a bright light background and via the microscope for haemolysis and / or agglutination). Degree of haemolysis was graded as follows: 3+ (complete haemolysis) and 2+ (partial >50% but not complete), 1+ (trace haemolysis), and negative (no visible haemolysis). Serum samples having a score of 3+ and 2+ were considered strongly haemolytic.

Data related to donor demographic characteristics such as age and gender was reported using descriptive statistics.Data were presented in tables and statistical analysis was performed using SPSS version 21.

Results

A total of 350 group O blood donors were screened for anti A and anti B titer and haemolysin. There were 215 males and 135 females. Malay was the predominant group (83%)The donor ages between 18 and 58 years with a median age of 24± 2.4. (Table 1)

About 52.9% of the donors have low titer (≤1:50) and 47.1% have high titer(≥1:100) of anti A and anti B. We observed that from 185 donors with low titer, male donors were predominant (n=133 vs. n=52 in female donors). Among female donors, 61.5 % have high titer antibody. The majority of donors with high antibody titer and low antibody titer came from the same age group, which is 18-30 year- old. (Table 2). Nineteen donors showed the reaction of ≥ 2+ in haemolysin test which gives the prevalence of haemolysins in group O donors was 5.4%. Anti A and anti B haemolysins were seen in 0.57% and 2.28%

of the donors, respectively, while donors having both A and B haemolysins in their sera was 2.57%. All 19 donors with haemolysin also have antibody titer of ≥ 1:100. The rest of the high titer donors (n=147) shown no haemolytic activity. (Table 3)

Discussion

The majority of our donors were Malay male (61%) ranged from 18 years to 58 years old with the mean age of 18-30 years old. Less female donors could be explained by blood donation criteria that excluding pregnant women, lactating women and menstruating women and also by the relatively low rate of women in high schools and universities (main places for our mobile collection) that full fill the general criteria's for blood donation in term of haemoglobin level and weight.

We observed about 53% of the donors have low titer (≤1:50) and 47% have high titer (≥1:100) of anti A and anti B. Our hospital takes cut off of ≤ 1: 50 as low titers and ≥1:100 as high titers for whole blood selection for EM O based on guidelines by our National Blood Centre. Previous study described high titer antiA/B antibodies was at least 64 and the prevalence of group O donors with high-titer anti-A and anti-B was 55.8% and 47.2% respectively [12]. Mc vey et al reported the majority of group O plasma (80%) had antibody titers of less than 1000 [13]. It was observed that lower titers to be more common with anti-A than anti-B haemolysins and ranged from 1:4 to 1:256 and 1:8 to 1:256, respectively[14].

We reported high titer and low titer were seen among donors at the age of 18-30 year old. This is because the majority of our donors were from this age and less donor beyond the age of 45 years old. However, previous study reported low mean titers were found in over 50-year-old men for anti-A in comparison with women of the same age. On the other hand, high mean anti-B titers were observed in young women (19 - 29 years old). High anti-A, B titers (>128) were observed in women of between 30 and 39 years old. Anti A, B titers< 32 were found in over 50-year-old men. Interestingly, they also observed that the lowest mean anti-B titers is for over 30-year-old men, and the highest frequency for anti-A > 128 is in over 30-year-old women [4]. One previous study in the Hainan province area reported high anti A and anti B titers among group O blood donors. They observed IgM antibody titers distributed in 4-1 024 and IgG antibody titer distributed in 2-2 048. Anti-A antibody titers of IgG were significantly higher than that of IgM anti-B, IgG anti-B, and IgM anti-A titers[15].

When we proceed to haemolysin test, not all donors with high antibody titers showed haemolytic activity. Haemolytic activity was seen in only 5.4% (n=19) of the donors, and all of them have antibody titer of ≥ 1:100. The rest of the donors (n=147) have high antibody titer but showed no haemolytic activity. Anti A and anti B haemolysins was seen in 0.57% and 2.28% of the donors, respectively, while donors having both A and B haemolysins in their sera was 2.57%. A study on Thai group O blood donors reported the higher prevalence of anti-A and anti-B haemolysins, which was 69%. Anti-A and anti-B haemolysins comprised 18.3% and 16.7%, respectively and 34% had both antibodies [16]. A previous study reported an overall higher prevalence of haemolysins in group O donors among Nigerian, which was 55.4%. Anti A only was seen in 10.3%, while Anti B haemolysin was seen in 12.6% of the donors. Donors having both Anti A and anti B haemolysins in their sera were 32.5%[17]. Lower prevalence of antibody was reported among blood donors in Lagos as compared to other studies. Only 15.4% had anti-A only, 5.1% had anti-B only, and 9.7% had both anti-A and anti-B haemolysins [14].

Immune ABO antibodies with haemolysin activity can be either of the IgM or IgG class and they are readily haemolised human red cell and able to agglutinate porcine A-like red cell. The complement-activating property of anti-A or anti-B may be more important than the titer, and hemolytic activity is not restricted to the IgM class alone [18]. The previous study reported from 51 of group O blood donors with a strong hemolytic sample, 62.8% had IgG titers of ≥ 64. There was a significant association between the grade of haemolysin and anti B IgG titer where they found that IgG titers remained high in a number of strongly haemolytic serum samples [19].

Previous study suggested that haemolysin test should be performed when group O whole blood or components containing plasma need to be transfused to non-group O recipients to identify the donors with high levels of anti-A and anti-B haemolysins [20]. It was reported that nearly 10% of transfused group O apheresis platelet had IgG titer ≥512, and 26.3% had ≥ 256. However, hemolysis due to incidental infusion of ABO incompatible plasma is known to be uncommon. They demonstrate the IgG antibody titer, in itself, is of limited predictive value. Other factors are likely to affect the risk of haemolysis i.e. transfusion volume and recipient [21].

It was demonstrated that the significant association among anti-A IgM titers, anti-A IgG titers and anti-A haemolysin grade. A significant association was also found between anti-B haemolysin grade and anti-B IgM titer [16]. However, Anti A haemolysin was reported to occur less frequently compared with anti-B haemolysin (Goran). Haemolysin test is a useful screening test to identify group O donors with high levels of anti-A and/or anti-B antibodies for safe blood transfusion [2].

We observed that low titer was seen predominantly among male donors (61.8% vs. 38.5% in female donors). The majority of female donors in our study showed high antibody titers (61.5 %). In general, there were more females than males with higher antibody titer levels, with significantly more females than males with anti-A, which may be caused by sensitization during pregnancies. [13, 16, 22]. High titers of anti-A haemolysins were reported to be associated with females [16]. However, previous study reported no statistically significant difference between male and female donors in the frequency of haemolysins [17]. Previous studies in Abidjan and Lagos also reported that age and gender showed no statistically significant on the frequency/presence of haemolysins [14, 23].

We observed that high and low titer donors come from age 18-30 years old, and this is because many of our donors were from this age group (51%). Previous studies among Thais and Nigerians reported anti-A and anti-B haemolysins were not associated with age and age has no significant impact on the frequency of haemolysins [16-17]. However, Study by de França et al. on their blood donors included all genders across all age groups, they found that ABO antibody titers decrease with age, with older males having the lowest titers by age 50. High mean anti-B and anti A, Btiters were observed in young donors [10].

Conclusions

Majority of our donors have low anti A and anti B titer, with only 5.4% had negative haemolysin activity. However, a significant percentage of group O donors have a high titer of anti-A or anti-B. The knowledge of the prevalence in our environment may suggest the need for routine screening in preparation for those emergency O whole blood that requires exchange transfusion in neonates with HDFN. Further studies to determine the type of antibody and severity of haemolysis are required to justify the clinical significance of such antibodies.

Conflict of interest

The authors declare that there is no conflict of interest.

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Ethical clearance

This study was approved by the Hospital Research and Ethical Committee.

Authors Contribution

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