CHARACTERISTIC OF ABO ANTIBODIES AMONG GROUP O BLOOD DONORS IN HOSPITAL UNIVERSITI SAINS MALAYSIA KELANTAN

BY

DR NUR ILYIA SYAZWANI SAIDIN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER IN PATHOLOGY (HEMATOLOGY)

SCHOOL OF MEDICAL SCIENCES

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MAIN SUPERVISOR:

PROF MADYA DR NOOR HASLINA MOHD NOOR

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LIST OF ABBREVIATIONS

DTT	Dithiothreitol
EDTA	Ethylene diamine tetra acetic acid
HTRs	Hemolytic transfusion reactions
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
JePEM	Jawatankuasa Etika Penyelidikan Manusia
PBS	Phosphate Buffered Saline
RBCs	Red blood cells
USM	Universiti Sains Malaysia

ABSTRAK

Latar Belakang dan Objektif: Tindak balas transfusi hemolitik akibat antibodi anti-A dan anti-B dalam produk darah kumpulan O jarang berlaku tetapi berpotensi membawa maut. Kajian ini bertujuan untuk mengenal pasti prevalens titer antibodi ABO yang tinggi dan kelas imunoglobulin sama ada IgM sahaja atau dengan gabungan IgG. Kami juga bertujuan untuk menganggarkan kelaziman titer IgG tinggi dan antibodi hemolysin dalam penderma darah kumpulan O dan untuk menentukan perkaitan parameter di atas dengan jantina dan umur.

Kaedah: Plasma daripada penderma darah kumpulan O telah diuji untuk titrasi antibodi ABO menggunakan teknik tiub konvensional pada suhu bilik, dan titer yang diperoleh lebih daripada 64 dianggap tinggi. Plasma kemudian dirawat dengan 0.01 M dithiothreitol (DTT) untuk menentukan kehadiran antibodi IgG dan titernya. Titer IgG lebih daripada 64 dianggap tinggi. Ujian hemolisis dilakukan menggunakan kaedah tiub dengan mencampurkan plasma dengan 3% suspensi sel A dan B segar dan diinkubasi pada suhu 37 darjah. Hemolisis diperhatikan secara makroskopik. Analisis statistik dilakukan menggunakan ujian Chi Square/Fisher Exact, dan nilai P kurang daripada 0.05 dianggap signifikan secara statistik.

Keputusan: Daripada 311 penderma, 238 (76%) menunjukkan titer antibodi anti-A dan/atau anti-B yang tinggi. Titer antibodi tertinggi yang diperolehi ialah 256. Titer anti-A dan anti-B yang tinggi didapati lebih banyak dalam kumpulan umur yang lebih muda (<40). Kami mendapati titer anti-B menunjukkan perkaitan dengan jantina (P <0.001), di mana penderma wanita (78%) menunjukkan titer anti-B yang tinggi. Daripada 311 penderma, 209 juga menunjukkan kehadiran jenis IgG antibodi ABO. Daripada jumlah ini, 124 (60%) penderma

mempunyai titer IgG anti-A dan/atau anti-B yang tinggi (≥64). Populasi kami menunjukkan prevalens hemolysin yang rendah iaitu 3.5%. Tiada perkaitan dilihat antara hemolysin dan titer antibodi**.**

Kesimpulan: Kajian kami menunjukkan prevalens tinggi penderma darah dengan titer antibodi ABO dan titer IgG yang tinggi, tetapi prevalens hemolysin yang rendah. Oleh itu, risiko hemolisis intravaskular mungkin tidak ketara. Walau bagaimanapun, kami masih mencadangkan titrasi antibodi dalam penderma wanita yang lebih muda kurang daripada 40 tahun untuk mengelakkan pemindahan produk darah O dengan titer yang tinggi kepada penerima bukan kumpulan O dan untuk memastikan produk darah selamat dapat diberikan kepada pesakit.

ABSTRACT

Background and Objectives: Hemolytic transfusion reaction due to anti-A and anti-B antibodies in group O blood products is rare but potentially fatal. This study aims to identify the prevalence of high ABO antibodies titer and the immunoglobulin classes either IgM only or with IgG combination. We also intended to estimate the prevalence of high IgG titers and hemolysin antibodies in group O blood donors and to determine the association of the above parameters with gender and age.

Methods: Plasma from group O blood donors was tested for ABO antibody titration using the conventional tube technique at room temperature, and titer obtained higher than 64 were considered high. The plasma was then treated with 0.01 M dithiothreitol (DTT) to determine the presence of IgG antibodies and their titer. IgG titers more than 64 were considered high. Test for hemolysis was done using tube method by mixing the plasma with 3% of fresh A and B cells suspension and incubated at 37 degrees temperature. The hemolysis was observed macroscopically. Statistical analysis was done using Chi-Square Test/Fisher Exact test, and P-values less than 0.05 were considered statistically significant.

Results: From 311 donors, 238 (76%) showed high anti-A and/or anti-B antibody titer. The highest antibody titer obtained was 256. High anti-A and anti-B titer were found more in younger age group (<40). We found anti-B titer shows association with gender ($P < 0.001$), in which female donors (78%) demonstrated a high anti-B titer. From 311 donors, 209 also showed the presence of IgG types of ABO antibodies. Of these, 124 (60%) donors have high

anti-A and/or anti-B IgG titer (≥64). Our population showed a low prevalence of hemolysins which was 3.5%. No association was seen between hemolysins and antibody titers.

Conclusion: Our study showed high prevalence of blood donors with high ABO antibodies titer and IgG titers, but low prevalence of hemolysins. Hence, the risk of intravascular hemolysis may not be significant. However, we still suggest antibody titration in younger female donors less than 40 years to prevent transfusion of high titer O blood product into the non-O recipient and to ensure safe blood products are given to the patient.

CHAPTER 1

INTRODUCTION

1.1 ABO Antibodies Titer, Immunoglobulin Classes and Hemolysins

The ABO blood group is most important among the 29 blood group systems. It was first discovered by Dr Karl Landsteiner in 1901. Using a simple experiment of mixing red blood cells (RBCs) and plasma from different individuals, he found a different agglutination pattern which led to the detection of three blood types: A, B, and O. Later in 1902, DesCasterllo and Sturli discovered the fourth type, AB blood group (1)(2). ABO antigens are one of the oligosaccharides antigens. These antigens are widely expressed on the membranes of red cells and tissue cells (epithelial cells, sensory neurons), as well platelets and endothelial cells. It is also found in secretions such as saliva and body fluid (3)(4).

Landsteiner law states that RBCs lack any of the ABO antigens, the person will always have the corresponding antibody (5). Thus, individuals of blood group O lacking A and B antigens will always have anti-A and anti-B antibodies (1). The ABO antibodies titers increased gradually in infants, with detection by serology at 8-month-old. The highest titers appeared at about 3-10 years old. Individuals above 80 years show decreased levels of ABO antibodies similar to 6-12-month-old infants (6). Among blood group O individuals, anti-A often have higher titer antibodies than anti-B (7).

Studies on ABO antibody titer among group O blood donors have been done worldwide. A study in the Indian population showed 57% and 51% of group O blood donors had titers $\geq$128 for anti-A and anti-B antibodies, respectively (8). More prevalence of high antibodies titer

(≥64) in group O blood donors was observed in Thailand, in which 76% and 80% of donors have high anti-A and anti-B titer, respectively (9). The Brazilian population showed that most blood donors have low antibodies titer, where 90.7% and 95.2% of donors have anti-A and anti-B antibodies titer <128 (10). The ABO antibody titer was also higher in females than in males' donors (8)(11). An earlier study comparing Asian, Caucasian, and Negro blood donors found a high titer of ABO antibodies in O group female Negro donors. The author suggests this was due to environmental influences such as parasitic infestation; Leishmaniasis, and Toxocariasis (12). In addition, a study by Mazda et al. found high ABO antibodies titers in younger female donors (less than 30) and female donors older than 50 years among the Japanese population (13).

The ABO antibodies were predominantly IgM, but a small proportion can be IgG or IgA (5)(14). Analysis of thirty-six cord blood samples in neonates with known ABO types revealed eighty percent of IgG antibodies, eleven percent of IgM antibodies, and six per cent of IgA antibodies (15). The large proportion of IgG antibodies in neonates is due to passive transfer from the maternal circulation. The half-life of these maternal IgG is three to seven weeks (15). The IgG antibodies found later are from the adaptive immune response after exposure to bacterial or food antigens (16). The presence of ABO IgM antibodies in neonates suggest a reaction towards utero infection or other immune stimuli (15). These IgM antibodies are also known as naturally occurring antibodies. It is produced after exposure to human microbes that have ABO blood group-like antigen (14).

Both ABO IgM and IgG antibodies can activate complement and mediate intravascular hemolysis. Antibodies with these properties are known as hemolysin (17). Studies on

hemolysin have been done worldwide. Khampanon et al. discovered a high prevalence of anti-A and anti-B hemolysins among Thailand blood donors. The anti-A and anti-B hemolysins were 18.3% and 16.7%, respectively (9). A study in Nigeria found a total of 30.3% of hemolysin. The anti-A and anti-B hemolysin was 15.4% and 5.1%, respectively, and 9.7% of donors have both hemolysin antibodies (18).

The anti-A hemolysin has been reported more prevalent than anti-B hemolysin among O group blood donors in Nigeria (18)(19) and Zimbabwe (20). Similarly, a study in the Indian population found 16.6% of anti-A hemolysin and 10.7% anti-B hemolysins from 187 group O blood donors tested (21). While a study in the National Blood Transfusion Center of Abidjan, Côte d'Ivoire, found donor with Anti-B hemolysins were more prevalent than anti-A hemolysins (15.71% of anti-B and 10.47% of anti-A) (17).

All the above studies also discovered that hemolysins antibodies were more prevalent in male donors. It was due to a more significant number of male than female donors. The low proportion of female donors could be explained by blood donation criteria that exclude pregnancy, lactation, and menses. There is no significant association found between the prevalence of hemolysins and gender, based on previous literature (17)(19) (22). However, among the female population, ABO hemolysin was found higher in pregnant than in non-pregnant women (23). These findings suggest a possible role of the fetus to stimulate anti-A and anti-B hemolysin production (22). No influence of age on the frequency of hemolysin antibodies have been reported (18)(19)(24).

The incompatibility between the donor's plasma and the recipient's red cell antigen can cause consequent red-cell destruction in the recipient. The incidence of hemolytic transfusion reaction (HTRs) following incompatible platelet transfusions is 1 in 9000 (25). These were seen in donors with high titers of ABO antibodies. A case of HTRs in group A recipient after receiving group O platelet apheresis containing high titer anti-A antibodies has been reported (26). This complication is seen more in platelet apheresis than random platelet concentrate due to the plasma volume differences (27). The suggested risk factors for hemolysis in an ABO-mismatched platelet unit recipient are high antibody titer, high volume of plasma infused, and infusion into a small blood volume recipient, such in neonate or child (7). Although rare, deaths attributed to hemolysis related to incompatible platelet transfusion have occurred (28).

In many transfusion centers, platelet inventories are generally inadequate. The adult patients are still transfused with mismatch group ABO platelets. Several strategies have been implemented to reduce the risk of HTRs following ABO-incompatible platelet transfusions. These include washing platelets, removing plasma by centrifugation, and combining plasma reduction with additive solutions or AB group plasma (29). However, such procedures may not eliminate the incidence of HTRs in donors with high titers antibodies. Moreover, the manipulation of blood components may cause bacterial contamination.

The ABO antibodies titration is the most common test used as a strategy to identify potential risk donors. Hence, in this study, we used anti-A and anti-B titration using the conventional tube method with a cut point titer ≥64 in plasma donor samples as a practical screening test

for “dangerous” O donors. The cut point of ≥64 was selected based on previous literature that used a similar cut of point titer in their study (8)(9)(29).

1.2 Justification of study

Blood group O components are the most available component in our transfusion unit. The transfusion of group O red cells or platelet concentrate into non-O-recipients is common practice here. These are seen in emergency cases such as trauma and obstetric bleeding. Blood group O red cells were also used for exchange transfusion in neonates. However, it has been recognized that group O transfusion is not entirely safe. Few cases of intravascular hemolysis after mismatch transfusion of O red cells and platelets have been reported (30) (31).

Hence, this study was carried out to determine the characteristic of anti-A and anti-B antibodies in our group O blood donors’. These characteristics include the ABO antibodies titer, immunoglobulin classes and hemolysin. We also want to determine the relationship between those parameters with gender and age. These data are helpful as a future reference in the transfusion practice and to ensure a safe blood product are supplied to the patients.

1.3 Dissertation Organization

This dissertation was arranged according to Format B (Manuscript ready format) according to the guideline by the Postgraduate Office, School of Medical Sciences (2016). The second chapter is the study objective. Chapter three is the manuscript that is ready for submission to the Asian Journal of Transfusion Science. The manuscript title is **“Characteristic of ABO antibodies in group O blood donors: The titers, immunoglobulin classes and**

hemolysin”. Chapter four is the study protocol submitted and obtained ethical approval from JEPeM, USM (JePeM code: USM/JEPeM/19120889). The last chapter contains an elaboration on methodology, the additional tables, and figures. The raw data was included in the attached CD.

CHAPTER 2

STUDY OBJECTIVES

2.1 General Objective

To determine the characteristic of anti- A, anti-B among blood group O blood donors.

2.2 Specific Objectives

1. To determine the prevalence of high anti-A and anti-B titers in group O blood donors and its association with gender and age.
2. To determine the prevalence of IgG type in anti-A and anti-B antibodies.
3. To determine the association of high anti-A and anti-B IgG titers with gender and age.
4. To determine the prevalence of anti-A and anti-B hemolysins in group O blood donors.

   All the above study objectives were included in the manuscript preparation.

CHAPTER 3

MANUSCRIPT

Original Article

Characteristic of ABO antibodies in group O blood donors: The titers, immunoglobulin classes and hemolysin.

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Abstract

Background and Objectives:

Hemolytic transfusion reaction due to anti-A and anti-B antibodies in group O blood products is rare but potentially fatal. This study aims to identify the prevalence of high ABO antibodies titer and the immunoglobulin classes either IgM only or with IgG combination. We also intended to estimate the prevalence of high IgG titers and hemolysin antibodies in group O blood donors and to determine the association of the above parameters with gender and age.

Methods:

Plasma from group O blood donors was tested for ABO antibody titration using the conventional tube technique at room temperature, and titer obtained higher than 64 were considered high. The plasma was then treated with 0.01 M dithiothreitol (DTT) to determine the presence of IgG antibodies and their titer. IgG titers more than 64 were considered high. Test for hemolysis was done using tube method by mixing the plasma with 3% of fresh A and B cells suspension and incubated at 37 degrees temperature. The hemolysis was observed macroscopically. Statistical analysis was done using Chi-Square Test/Fisher Exact test, and P-values less than 0.05 were considered statistically significant.

Results:

From 311 donors, 238 (76%) showed high anti-A and/or anti-B antibody titer. The highest antibody titer obtained was 256. High anti-A and anti-B titer were found more in younger age group (<40). We found anti-B titer shows association with gender (P <0.001), in which female donors (78%) demonstrated a high anti-B titer. From 311 donors, 209 also showed the presence of IgG types of ABO antibodies. Of these, 124 (60%) donors have high anti-A and/or anti-B IgG titer (≥64). Our population showed a low prevalence of hemolysins which was 3.5%. No association was seen between hemolysins and antibody titers.

Conclusion:

Our study showed high prevalence of blood donors with high ABO antibodies titer and IgG titers, but low prevalence of hemolysins. Hence, the risk of intravascular hemolysis may not be significant. However, we still suggest antibody titration in younger female donors less than 40 years to prevent transfusion of high titer O blood product into the non-O recipient and to ensure safe blood products are given to the patient.

Keywords: ABO**,** Antibody titers, Dithiothreitol, Hemolytic transfusion reaction, O group, Hemolysin, IgM and IgG titers.

3.1 Introduction

In Malaysia, blood group O was the highest among Malays and Chinese (36.7%). In Indian donors, blood group O and blood group B were of equivalent frequencies (1). Blood group O is well known as the universal blood group. It is due to its red cells lack of ABO antigen (2). Thus, it is widely accepted as safe to transfused group O red cells to non-O group recipients, particularly in life-saving conditions (3).

However, its plasma contains anti-A and anti-B of IgM type. These antibodies are naturally occurring because they are produced without exposure to red blood cells (4). A small proportion of ABO antibodies can be IgG or IgA. These antibodies arise following immunization or naturally occurring (5). The production of ABO antibodies starts at birth, but the titer is usually too low for detection. The antibody levels increase at three to six months of age, with peak production between five to ten years and declines later in life. Older people over 65 years old usually have lower anti-A, anti-B antibodies that may be undetectable on reverse blood grouping (6). Among blood group O individuals, anti-A titers are often higher than anti-B titers (7).

A study among the Indian population found 57.12% and 51.9% of O blood donors had titer ≥128 for anti-A and anti-B, respectively (8). In the Brazilian population, only 9.29% of anti-A and 4.81% of anti B from blood group O donors have titer ≥128 (9). These findings suggest the possibility of influence from the genetic background on the antibody's titer. In both studies, the authors demonstrate a high titer in female donors, with an inverse relation between antibodies titer and age, as agreed by previous literature works (6)(10).

The ABO IgM antibodies ≥ 64 have been agreed in many literature as high titer, while high titer for IgG antibodies is ≥ 200 (8)(11)(12)(13). During the Vietnam War, the military force used a low titer of whole blood O for treating hemorrhagic shock. The US military's low titer threshold during the war was less than 256 (14). United Kingdom implements antibody titration for all O group donors where ABO antibodies titer >100 was considered high titer and allowed transfuse to only O blood group patients (8). However, there is a lack of standard guidelines in defining the high titer of ABO antibodies among the transfusion community. Our study uses a cut point titer of ≥64 as a high titer for both ABO IgM and IgG antibodies. Both ABO IgM and IgG antibodies can activate complement and result in intravascular hemolysis (15). These may occur following the transfer of ABO antibodies into non-O recipients, such as in ABO-incompatible platelet transfusion (16).

This study aims to estimate the prevalence of high titer of ABO antibodies in our local population and its association with gender and age. We also aimed to determine the type of immunoglobulin of anti-A and anti-B antibodies, either IgM only or with a combination of IgG types. We also intended to identify the prevalence of high IgG titer and hemolysin antibodies among our group O blood donors. The group O blood product is widely used in clinical transfusion practice. Thus, the finding in this study may also add to the maintenance policy of safe transfusion practice in transfusion therapy. To the best of our knowledge, no similar research has been performed in our local population.

3.2 Material & Methods

Study setting

The study was conducted at Transfusion Medicine Unit of Hospital Universiti Sains Malaysia from January to December 2020. The study protocol has been approved by the Institutional Review Board of the Human Research Ethics Committee (JePeM code: USM/JEPeM/19120889).

Study population

A total of 311 samples were collected randomly from whole blood group O donors, from 18 to 60 years old. All selected donors have fulfilled the pre-donation criteria as per national guidelines for donor selections.

Sample and reagents

About 3 ml of blood was collected into the Ethylene-diamine-tetra acetic acid (EDTA) tube. The sample was stored in a 4-6-degree freezer and analyzed less than 48 hours after collection. In house preparation of 3% concentrated A cells and B cells was used for titer studies, immunoglobulin, and hemolysis test. A 0.01 M of Dithiothreitol (DTT) (SIGMA-ALDRICH D9163) was used to cleave disulphide bonds of pentameric IgM. DTT will eliminate IgM's agglutinating reactivity and permit the detection of IgG antibodies in the plasma (17).

Titration procedure

Titration procedures were done to assess anti-A and anti-B titer as per method described by Technical Manual American Association of Blood Bank (AABB), sixteenth edition (18).

Plasma sample mixed with 0.9% saline into the glass tube and two sets of serials 2-fold doubling dilutions were prepared. One drop of A1 cells and B cells suspension was added to the diluted plasma, respectively. The test mixture was incubated at room temperature for 10 minutes and centrifuge at 3400 rpm for 15 seconds. The highest dilution causing agglutination (macroscopic observation = 1+ reaction) was assumed to represent IgM antibodies titer (19). The antibody titer of more than 64 was considered high.

Determination Immunoglobulin classes

The presence of IgG was detected by comparison reactivity of treated plasma with Dithiothreitol (DTT) and untreated plasma in Phosphate Buffered Saline (PBS) using glass tube. Serial dilutions were prepared from treated plasma with DTT, the mixture then incubated for 60 minutes at 37 degrees. The serial dilution of untreated plasma in PBS was incubated for 30 minutes at 37 degrees. Four drops of each dilution from treated and untreated plasma mixed with one drop of A1 cells and B cells into new set of tubes, before proceeding with centrifugation. The agglutination observed for each dilution were graded. Equivalent reactivity in both treated and untreated plasma indicates the presence of the IgG antibodies. The IgG titers represent by the highest dilution with observe agglutination, and a titer of more than 64 was considered high. Lack reactivity in the treated plasma and reactivity in untreated plasma indicates only IgM antibodies. The procedure and interpretation were adapted from the Technical Manual American Association of Blood Bank (AABB), sixteenth edition (20).

Hemolysin Test

For hemolysin test, 100 microliters of plasma were dispensed into tube labels A and B. Another 100 microliters of A1 cells and B cells were added to the tube labeled A and tube labeled B, respectively. The mixture then incubated at 37°C for 60 minutes, followed by centrifugation. The presence of hemolysis was observed macroscopically according to Clinical Laboratory Standard Institute guidelines. Negative hemolysis was confirmed by spectrophotometry measurement, defined by free hemoglobin in plasma less than 0.5 g/L (21). The procedure for hemolysin test was done as per standard procedure (22).

Statistical analysis

Descriptive statistics were used to describe gender, age, ethnicity, antibodies titer, immunoglobulin classes and hemolysin. The statistical analysis was performed using the Pearson Chi-Square Test or Fisher Exact Test to determine the association of antibody titer, hemolysis, with gender and age. P value less than 0.05 was considered statistically significant. All data were analyzed by standard statistical software (SPSS version 25).

3.3 Results

Demographic data from 311 group O blood donors selected shows 185 (60%) were males, and 126 (40%) were female. The largest ethnicity is from Malay 273 (88%), followed by Chinese 33 (10.6%), Siamese 4 (1.3%) and Indian 1 (0.3%). For the age group, the 18–29-years contribute to the highest number of donors, 144 (46%), followed by 30-39 years 92 (30%), 40-49 years 43 (14%), and ≥ 50 years old is 32 (10%). The mean age of our study population is 32.5-year-old.

Anti-A, Anti B titers

From 311 donors, 238 (76%) donors have high anti-A and/or anti-B titers (≥64). Donor with high anti-A titer only was 32 (10%), high anti-B titer only was 26 (8%), and high anti-A and anti-B titer was 180 (58%). There were slightly more donors with high anti-A titer than anti-B titer (**Table 3.1)**. The means, median and range of anti-A and anti-B titer was shown in **(Table 3.2)**. The highest antibody titer obtained was 256 for both anti-A and anti-B antibodies.

We found that most female donors showed high anti-A and anti-B titers. From 126 female donors, 90 (71%) showed high anti-A titers, and 98 (77%) showed high anti-B titers. Of these, they may have a high titer for anti-A or anti-B alone or a high titer for both antibodies. Among female donors, high antibodies titer was found more in younger age (<40). Female donors also have high mean antibodies titers compared to male donors **(Table 3.3)**. Of 185 male donors, 122 (66%) have high anti-A titers, and 108 (58%) have high anti-B titers. Similar to females, the high antibodies titer mainly was seen in less than 40 years old male donors **(Table 3.4)**.

We studied the relation between ABO antibodies titer with gender and age. There is no statistical association between anti-A titer and gender. However, anti-B titer shows association with gender ($P < 0.001$), in which female donors (78%) demonstrated a high anti-B titer **(Table 3.5).** We found no correlation between the antibody titer with age.

Immunoglobulin classes

We found some donors have combination of IgM and IgG types of ABO antibodies. From 311 donors, a total of 209 donor has IgG types of antibodies. Of these, 46 (15%) donors showed presence of anti-A IgG and 45 (14%) donors showed presence of anti-B IgG. While 118 (38%) donors have IgG for both anti-A and anti-B antibodies. The remaining 102 (33%) donors have only IgM type of ABO antibodies **(Table 3.6).**

Anti-A, Anti-B IgG titers

From 209 donors with IgG types of antibodies, we found 124 (60%) donors have high IgG titer (≥64) **(Table 3.7)**. The IgG titer ranges from 32 to 256. The high IgG titers were mainly seen in younger group donors, less than 40 years. We found significant association between anti-B IgG titer with gender (P = 0.002), in which female donors have high chances of obtaining high anti-B IgG titers **(Table 3.8)**. There were no association seen between anti-A IgG titer with gender, and no significant association seen between anti-A IgG and anti-B IgG titer with age.

Hemolysin antibodies

A total of 11 donors (3.5%) were found to have hemolysin antibodies **(Table 3.9).** Most donors with hemolysin were males and were less than 40 years old. Anti-B hemolysins were more prevalent than anti-A hemolysins and were mainly found in male donors. In contrast, female donors have more prevalence of anti-A hemolysin. Most donors with hemolysins also have high antibody titers and IgG types of immunoglobulins. The details of 11 donors with hemolysin are shown in **Table 3.10**. There was no statistically significant association between prevalence of hemolysins with gender and age. Crosstabulation between IgM titer,

and IgG titer of anti-A and anti-B antibodies with hemolysis also shown no significant association.

3.4 Discussion

It is known that blood group O containing high anti-A and anti-B titer could lead to hemolytic transfusion reactions (HTRs) after ABO-incompatible blood transfusion (23). A case of HTRs after mismatch transfusion with O platelet apheresis with high titer of anti-A antibodies has been reported (24). HTRs also has been seen following transfusion of group O red cells to group A recipient (25). These findings suggest a mismatch transfusion of blood group O is not entirely safe, and hemolysis may be more common than appreciated.

Our study noticed a high prevalence of donors with high anti-A and anti-B titers. A recent study in the Indian population found the prevalence of donor with high anti-A and anti-B titer was 36% and 32%, respectively (26). In Thailand, more prevalence of high ABO antibodies among group O blood donors was observed, in which 76% and 80% of donors showed high anti-A and anti-B titer respectively (19). The Brazilian population showed that most blood donors have low antibodies titer, where 90.7% and 95.2% of donors have anti-A and anti-B antibodies titer <128 (9). A study in Korea on the platelet apheresis O donor found 50% of group O components were labeled as high titer at dilution 1 in 150 and 25% at dilution 1 in 250 (27). A study among group O male donors in South Texas found from 3274 donors, 426 (13%) have high titer antibodies using cut point of 256 (28). Josephson et al. found 28% from 100 group O platelet apheresis samples have a high anti-A and anti-B titers, which range from 64 to 256 (13). A study on group O pooled platelet concentrate in

Michigan, United States, found approximately 60 percent of group O platelets have high-titer antibodies (≥64) (12).

Our study discovered titer range from 16 to 256 for both anti-A and anti-B antibodies. In comparison, Khampanon et al. discovered anti-A and anti-B titers ranging from 8 to 1,024 among group O blood donors ((19). Neelima et al. found even higher antibodies titer ranging from 2 to 4096 in Indian donors using a similar tube titration technique (29). A study among O blood donors in Brazil found ABO antibodies titer ranging from 16 to 128 (9). The antibody titer ranges from 4 to 128 for anti-A and anti-B antibodies among Korean blood donors using the tube method (30). In comparison, Laura et al. found both anti-A and anti-B titer range from 4 to 512 using the gel method among group O blood donors in United States (12).

We studied the gender-wise distribution of titers and discovered high mean antibodies titer in female than male donors among all age groups. Our study also showed high antibodies titer in younger females less than 40 years old. This is similar to study by Sujitha et al., in which they found high titer of anti-A titer among 30-39 years group (26). This is possible due to sensitization by immunization and pregnancy in this reproductive age (31). Females can develop stronger humoral and adaptive immune responses than males, thus generating higher antibody levels (32). These are marked by higher basal and post-vaccination IgG levels and increased B cell numbers in response to vaccination and viral infection (33). The chromosomal genetic differences, sex hormones, and gender-specific differences in the microbiome are among the factors that influence the difference in humoral immunity between males and females. The X chromosome expresses ten times more genes than the Y

chromosome, and many genes on the X chromosome are known to influence immunity, thus affecting antibody production (32).

We studied the age-wise distribution of titers in O group donors. The mean age of the donor is 32 years old, and 46% of blood donors belong to the 18-29 years old group. The high mean titer was found in donors less than 40 years old. These findings are similar to a report from India in which they found a higher mean titer for the younger age group (8). We also found a low mean titer in male donors ≥50 year's old, similar to the findings of de Franca et al., In addition, few study among Indian donors showed consistent findings of reduced titer with increasing age (8)(26). However, a study in the Korean population found a low median titer of 16 for anti-A and anti-B across all age groups (30). A recent study in Japan demonstrated that age was a suppressor of antibody responses. They revealed that the antibody titer after mRNA SARS-CoV-2 vaccine significantly reduces as older (34). During aging, T- cells favor short-lived Effectors cells but not Memory T-cells or T-Follicular Helper responses, thus affecting B cells for the generation of antibodies (35).

The ABO antibodies titers were also influenced by ethnicity, diet, lifestyle, and environmental factors (36). The high titer of ABO antibodies in Asian and African has been suggested due to mosquito bites and parasitic intestinal infections (37). The Asian country is known to have a high percentage of forest areas with increased susceptibility for mosquito bites. The high titers of ABO antibodies can also be due to vaccination or following antigen exposure, such as pregnancy or blood transfusion (38). Gupte et al. found immunization with tetanus toxoid boosts anti-A and anti-B titer levels in pregnant women and normal adults. The increment of antibody titers was observed in 20 per cent after one injection, 40 per cent

after two injections and 60 per cent after three injections. (31). A rise in antibodies is more in anti-A due to A-like substance in hog stomach pepsin used in toxoid production (39).

Lifestyle changes also have been reported to associate with variation in titer levels. An apheresis donor with a history of consumption of probiotics for three years was found to have high anti-B titers and cause HTRs to the group B recipient. The probiotic formulations contain bacteria with B-like antigens that stimulate increased anti-B production when ingested by individuals lacking the corresponding antigen (39). Probiotics also have been shown to boost antibody responses to salmonella and rotavirus oral vaccine (40). Study by Sujitha et al., comparing type of diet and high titer antibodies found a significant association (P=0.018). Donor with vegetarian types of diet have high chances to get high titer antibodies (61%) compared to mixed type of diet (48%) (26). A study in the Japanese population revealed a decrease of anti-A and anti-B titer over 15 years (10). Titer reduction is due to the improvement of the environment and self-hygiene that lead to less parasitic infestation and enteric bacterial infection (8).

Our study showed many of our blood donors also have a presence of IgG in their ABO antibodies. In addition, we found that most of them have a high IgG titer similar to the Thailand population. Among Thai blood donors, high anti-A and anti-B IgG titer was 93.0% and 95.3%, respectively (19). Our study also revealed that female donors have a greater chance to obtain high anti-B IgG titer. With this, one expects to observe a more incidence of HTRs if given blood product from a female donor. A high titer in the female group is due to sex hormone influence on immunity. Estradiol, at physiological concentrations, can stimulate antibody production by B cells, suggesting one possible mechanism mediating

higher antibody production in females (33). The high IgG titers in our study were mostly seen in the younger age donors. In comparison, Japanese O blood donors showed high anti-A and anti-B IgG titers with increased age in females group (10).

The prevalence of ABO hemolysin antibodies in our study is 3.5%. This result is lower than those reported by Khampanon et al., who had a prevalence of 69% in Thailand group O blood donors (19). In India, the prevalence of anti-A hemolysin was 16.6%, and anti-B hemolysin was 10.7% in group O blood donors (37). A high prevalence of hemolysin has been reported in Nigeria based on a few studies; 30.3% (39), 35% (40) and even higher prevalence of hemolysin (52.8%) had been found (41). Despite the high titer of ABO antibodies that we found, yet the hemolysin frequency is very low. In comparison, in study by Olawumi and Olantuji, they noticed hemolysis at titers as low as 8 (42). Bastos et al. also observed hemolytic sera in donors with low anti-A and anti-B IgM titer (≤64), 21.9% and 12.8%, respectively. (43). These findings suggest that ABO antibodies titer does not always correlate with hemolysis.

We observed anti-B hemolysins are more frequent in male donors, with their anti-B titer range from 32 to 256. But female donors showed more frequent anti-A hemolysins with their anti-A titer range from 64 to 128. Khampanon et al. found among Thai blood donors, the high titers of anti-A hemolysins were associated with females ($P < 0.05$). Our studied showed high prevalence of ABO hemolysin in the younger group for both male and female donors (<40 years). A study in National Blood Transfusion center of Abidjan, Cote d'Ivoire also found significant age distribution of ABO hemolysin among 25-29 years blood donors ($P = 0.001$) (44). Among our blood donors, the titer for anti-A hemolysins were higher than anti-

B hemolysins. These findings are comparable to the study in Zimbabwe and Nigeria, in which their titer for anti-A hemolysin was higher than anti-B hemolysin (42)(45).

Majority of our blood donors with hemolysin also have high IgM and IgG titer. James et al., in their study, also showed that high anti-A and anti-B IgG titer (≥64) were present in more than 60% of the hemolytic sera (45). A study in the Indian population showed that from 51 hemolytic sera, 32 (62.8%) had high lgG titers (≥64). This was postulated due to tetanus toxoid injections as 81 per cent of blood donors reported having taken it, and 38 per cent of them donated within one year of immunization (37). We found no association between IgM and IgG titer with hemolysis. However, a recent study found a good correlation between high anti-A and anti-B IgM titer (>64) with hemolysis (43). The high titer of IgM and IgG antibodies can cause complement-mediated red cells lysis (46). The IgM antibodies can bind to C1q and initiate complement cascade, leading to membrane attack complex formation and consequent cells lysis. The IgG antibodies, particularly the IgG1 and IgG3 subclasses, also can fix complement efficiently, thus causing mild to fatal HTRs (43) A recent study by Bastos et al. found IgG1 and IgG3 types of ABO antibodies were correlate well with hemolysis (P= < .001).

The limitation of our study is we used conventional tube titration to detect high ABO antibodies titer. Inter-laboratory variations were seen using this method (47)(48). This variation is due to differences in medium use for dilution, incubation time, centrifugation speed and time. More sensitive techniques such as titration by gel hemagglutination, flowcytometry, or automated technologies are available nowadays. A study in Stanford blood center, California comparing ABO antibodies titer between automated and manual

tube method found (15%) of platelet concentrate have high antibodies titer (>256) using automated microplate system than (12%) by manual method (49). Attempt to standardize ABO titers with an automated instrument led to an opportunity to use standardized samples and medium, either gel-based or solid phase-based and help identify blood products containing high antibody titers effectively.

3.5 Conclusion

In conclusion, our group O blood donors showed a high titer of ABO antibodies, high titer IgG antibodies but a low prevalence of hemolysins. The HTRs after mismatch transfusion of platelet/red cells group O is expected to be low in our population. However, close monitoring is needed when transfusing incompatible platelet as HTRs can be clinically subtle (46)(50). We recommend for transfusion unit to screen ABO titer for younger female group O donors routinely; by single plasma dilution with cut point titer 64 as a screening tool. Hence, any blood products with high titer should be labeled and transfused only to O group patients.

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