ABO Blood Group Antibody Titre in Individuals at Different Ages; Clinical Significance & Future Scopes in Transfusion Medicine

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Abstract: Aim: Here in this study we are focusing on antibody titre level of different age groups, and discuss its importance in various clinical scenarios. Objectives: To find out the Anti A, Anti B titre levels in A, B and O blood group individuals, the pattern of titre level, and to look for any gender predominance and discuss clinical significance of antibody titre level in various aspects of transfusion. Method: The study were performed on a total of 300 blood samples received in Blood Bank at St. John’s medical College Hospital, Bangalore for blood grouping and typing and donor samples received for cross matching. Samples were categorized into 4 groups: 30 samples collected from the age group of 5 months to 1 year and 90 samples each collected from rest of following groups: 5-10 years, 20-25 years and 60-65 years. Results: In our study we concluded that the naturally occurring ABO antibodies begins to synthesize at the age of 5-6 months will reach its peak level by the age of 20-25 years and gradually decrease as the age advances. There is no significant difference in the levels of Anti A and Anti B levels of ‘A’ and ‘B’ group & Anti A, Anti B levels in ‘O’ group individuals. Titre levels of both the antibodies seem to show similar values in both the genders. Conclusion: Various transfusion requirements like, pregnancy, incompatible transfusion due to unavailability of compatible group, plasma apheresis, plasma exchange, incompatible platelet transfusion, ABO incompatible organ transplantation require thorough monitoring of naturally occurring Antibodies, So we suggest that Compulsory antibody titration of all donors, especially O group donors (as they produce IgG type antibodies) should be done.

Keywords: Naturally occurring ABO Antibodies, Haemolytic Disease Of New Born, ABO incompatible transplantation, Plasmapheresis, Antibody Titre

1. Introduction

The ABO system is the most important of all blood groups in transfusion practice. It is the only blood group system in which individuals predictably have antibodies in their serum to antigens that are absent from their RBCs. The ABO blood group system was initially identified because of the finding that naturally occurring antibodies are always found in blood plasma. Although not all blood group antibodies are clinically significant, they do play a crucial role in transfusion medicine, in terms of the practice of blood transfusion. [1] Clinically significant antibodies have the potential to result in transfusion-related adverse effects ranging from mild to severe as well as hemolytic illness in foetuses and newborns after placental transfer from the mother to the foetus. Mode of reactivity and historical data on specificity play a significant role in determining the clinical importance of antibodies. [2] Anti A is present in B group individuals, anti B is present in A group individuals. Anti A and anti B present in O group individuals. They are IgM in nature and correspond for the immune response. Antibodies generally begin production within 3 months of age. The titre of anti A, anti B reaches the maximum at the age of 5-10 years, remains constant in adult life and declines thereafter. In this study, the antibody titre of anti A and anti B in individuals of different blood group in specific age groups are evaluated. [3] Humans produce natural antibodies which are present in serum before any infection. Natural antibodies have been defined as antibodies that are produced without any previous infection, vaccination, other foreign antigen exposure or passive immunization. Anti A and anti B antibodies which are not present in the newborn, appears in the first few years of life. They are isoaotibodies, that is they are produced by an individual against antigen produced by members of the same species (isoantigens). Anti A and anti B antibodies are usually IgM type which are not able to pass through the placenta to the foetal blood circulation. O type individuals can produce IgG type of ABO antibodies. These natural antibodies are directed against the disaccharide galactose-a (1-3) galactose, which is found as a terminal sugar on glycosylated cell surface proteins, and generated in response to production of this sugar [4]. It is possible that food and environmental antigens (bacterial, viral or plant antigen) have epitopes similar enough to A and B glycoprotein antigens. These antibodies created against these environmental antigens in the first years of life can cross react with ABO incompatible red blood cells that it come in contact with during blood transfusion later in life [5]. It can provide information to compare the strength of antigen expression on different cell samples. Antibody titers are useful in prenatal evaluation of hemolytic disease of the fetus and newborn (HDFN) [6]. In this study titre levels of anti A and Anti B in A, B and O groups were measured and its relevance in various transfusion aspects are discussed.
2. Review of Literature

It is difficult to prevent hemolysis caused by the transfusion of platelets (PLTs) that are incompatible with ABO plasma. The transfusion field has not come to an agreement on critical antibody titers. In order to comprehend the trends of anti-A and anti-B antibody titer levels in O group donors and to spot any distinct patterns of distribution in connection to age and gender, Anita amar et al done a study and they found that 57.12% and 51.19% of all donors had titers ≥128 for anti-A and anti-B, respectively. The geometric mean of anti-A and anti-B was 155.7 and 137.28, respectively. The titers were significantly higher (P < 0.001) in female donors. An inverse relation between titer levels and age was seen.

Another experimental study was done where they investigated acute and delayed red blood cell (RBC) antibody-associated complications, including haemolysis, in major and bidirectional ABO incompatible Haematopoietic stem cell transplantation and their results confirmed that the occurrence of haemolysis depends on larger RBC volumes and higher isoagglutinin titres. Platelet transfusion with high ABO antibody titres can cause risk of hemolysis if the unit crosses the ABO type, on this Gayathri et al done a comparative study on ABO antibody levels in apheresis platelets suspended in platelet additive solution (PAS) and plasma and they concluded that The median ABO antibody titres were lower in Apheresis Platelets suspended in PAS than in plasma. Addition of the PAS significantly lowered the IgM antibody titres by twofold, compared to plasma. Susceptibility to Covid-19 has been found to be connected with the ABO blood group, with type persons being at a lesser risk. The fundamental mechanism hasn't been clarified, though. In a study by Marie dellers et al, they have intended to explore the hypothesis that Covid-19 patients would have lower levels of ABO antibodies than non-infected persons since they could offer some degree of protection. A study by Noor Haslina Mohd et al aimed 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 and they have suggested that in a large portion of group O donors, Anti-A or anti-B titers are high. Hence, despite the labour intensiveness of haemolysis titration method and the regular exchange transfusion of group O blood, there is a requirement to. We regularly check our donors for haemolysins to determine which ones provide the biggest risk to recipients. There was a significant association between maternal anti-A/B IgG titer and hyperbilirubinemia requiring treatment according to the study done by Greth et al.

3. Aim

Here in this study we are focusing on antibody titre level of different age groups, and discuss its importance in various clinical scenarios.

4. Objectives

To find out the Anti A, Anti B titre levels in A, B and O blood group individuals, the pattern of titre level, and to look for any gender predominance and discuss clinical significance of antibody titre level in various aspects of transfusion.

5. Materials and Method

5.1 Source of Data

The study were performed on a total of 300 blood samples received in Blood Bank at St. John’s medical College Hospital, Bangalore for blood grouping and typing and donor samples received for cross matching. Samples were categorized into 4 groups.30 samples collected from the age group of 5 months to 1 year and 90 samples each collected from rest of following groups.5-10 years, 20-25 years and 60-65 years.

5.2 Exclusion criteria

Patient who received blood transfusion in the past one month and patients with blood group AB are excluded as they don’t possess antibodies in the serum.

5.3 Sample Processing

Samples can be stored between 2-8°C for 2 days, or frozen at-30°C for 1 year. Blood specimens exhibiting gross haemolysis or contamination were not used.

5.4 Method

Serial Dilutions, 1: 2 dilutions until 512 dilutions of serum with 2-5% normal saline done on each sample and corresponding commercial red cell reagents were added, After 1 hour of incubation at room temperature all the tubes along with control (undiluted serum+Red cell reagent) are centrifuged. Agglutinations are observed microscopically and the titre is recorded as the reciprocal of the highest serum dilution in which there is agglutination. Grade and record results. Report the highest dilution that gives 1+ macroscopic agglutination which will be the endpoint. Antibody titration were performed by using tube technique. Other methods like tube technique, gel card technique, can also be used. Grading agglutination reactions gives an indication of the relative amount of antigen or antibody present, all tubes and should be graded.

6. Result

In the first age group of babies, 30 samples were analyzed, 10 samples each from A, B and O group. And in all other age group 90 samples were analyzed.30 samples each from A, B and O group.

Here the average of titres i.e., average of anti A from B group individuals and anti B from A group individuals and both anti A and anti B from o group individuals were calculated for each categories.
In this age group total 30 samples were analyzed, 10 each from A, B and O group. Anti A titre was taken from B group and O group individuals. Anti B titre was taken from A group and O group individuals. In most cases, anti A and anti B shows agglutination in 1: 8 dilution. The mean of anti A titre was 15 and mean of anti B titre was 18.2 as shown in figure 1.

In this age group total 90 samples were analyzed, 30 each from A, B and O group. The mean of anti A titre was 129.8 and mean of anti B was 141.8.

In this age group most individuals shows agglutination in 1: 128 dilution.

In the age group of 20-25 years which are the healthy age group shows the high value of titre as compared to the other age groups. The average value of anti A was 351.9 and value of anti B titre was 327.1.

In this age group also most individuals shows agglutination in 1: 128 dilution.

In this age group of elderly the average value of anti A titre is 140.5 and anti B is 136.2 The average value is less when compared to the healthy age group. In this age group most of the individuals shows agglutination in 1: 64 dilution. And most of them come in the range of 32-128.
7. Discussion

In this study the antibody titre was done in different age groups. The study reveals that the antibodies are present in lower level in infants and the value increases by advance in the age. The titre value is highest in 20-25 year age group and gradually the value of titre declines when it comes to the older age group.

Immunoglobulin synthesis starts in utero after about 20 weeks of gestation. The quantity and quality of the stimulations are such that only IgG and IgM responses are evoked. In the cord serum, IgM is routinely present and evidence indicates that IgG synthesized by the baby is probably or routinely present, although in low amounts [13], [14]

Theodore Thomaidis et al, did antibody titre in 250 full term infants, in 148 newborns anti A or anti B or both saline active isoagglutinins were detected [10]. Similarly in around 60% of full term infants natural isoagglutinins were found in the study done A. Agathopoulos et al. [15]

In another study by Allansmith et al, also they concluded that IgG synthesis by infant probably begins at from 4-6 weeks of age. IgM synthesis begins in the first few days of life and the system expands to the adult level at about 1 yr [16]

Aubert et al found that the commonest anti A titre was 256 and the commonest anti B titre 64. The developmental pattern of ABO saline isoagglutinins were determined in 272 children between birth and 16 yrs of age in the study done by W. F Taylor et al, and the anti B titre was lower than that of Anti A titre [17]. In the present study there is no significant difference in titres of anti A and anti B. Though in this study the titre was not done in newborns, but in the infants (6-12 months) had already developed antibodies (average titre 1: 8) which increased to 1: 32 by 1-5 years of age concluded by further studies.

Thomsen kettel found anti A titre to be higher in group O subjects than group B subjects. But no similar difference in anti B titre of A group and O group subjects. Ichikawa also found that titre of Anti B to be higher in O group than in group A subjects. However in the present study there is no significant difference in the anti A and anti B of O group and A and B group. The amount of anti A and anti B in the serum may be influenced by the sex of the individual. Study done by Nijenhuis et al tells that anti B titre was significantly higher in females. We couldn’t find out any significant difference of antibody titre in both genders. The study reveals that at the antibodies are present in lower level in infants and the value is increased by the age 1-5 years. The titre value is highest in 20-25 year age group and gradually the value of titre declines by the age of 60. There is no significant difference in the anti A and anti B of O group and A and B group.

8. Conclusion

In our study we concluded that the naturally occurring ABO antibodies begins to synthesize at the age of 5-6 months will reach its peak level by the age of 20-25 years and gradually decrease as the age advances. There is no significant difference in the levels of Anti A and Anti B levels of ‘A’ and ‘B’ group & Anti A, Anti B levels in ‘O’ group individuals. Titre levels of both the antibodies seem to show similar values in both the genders.

The relevance and clinical significance of determining the antibody titres comes when there is

1) Transfusion in pregnancy-The risks are high in case of ABO HDN as the Anti A and Anti B in “O” group individuals are IgG in nature and they are able to cross the placenta and cause serious adverse problems. Their Titre level monitoring is crucial as the pregnancy advances. The treatment and management will based on the titre levels.

2) ABO plasma-incompatible PLT infusions-Significant levels of ABO antigen are present on the surface of Platelets, and anti-A and anti-B antibodies are present in
plasma. The PLT units are transfused ABO-identical if available, but if the transfusion is with the unidentical blood group, the Platelet units with high ABO antibody titres can pose the risk of hemolysis. This danger is increased when group O Platelets are transfused to non-O patients. The severity will increase as the titre of antibody increases.

3) In ABO incompatible haematopoietic stem cell transplantation, as well as in other incompatible organ transplantation the extend of haemolysis depends on the high titer of antibodies.

4) During Platelet Apheresis-When a considerable volume of incompatible plasma is transfused along with the platelets, apheresis platelets suspended in the PAS should be taken into consideration since the risk of hemolysis owing to passively transfused anti-A and anti-B is minor but present. Contrary to apheresis platelets suspended in plasma, storing apheresis platelets in Platelet additive solution lowers IgM ABO antibody titre levels by twofold.

To conclude, there is the need to routinely screen our donors for haemolysins in order to identify those posing the greatest risk to recipients. Determining antibody titre in indiduals will help in reducing transfusion related risks in blood transfusion practices especially in ABO-incompatible plasma and platelet transfusions and ABO-incompatible transplantsations.

9. Future Scope

Antibody Titre must be done in all donors especially in O group donors as they are IgG in nature to avoid troublesome experiences while transfusion in cases of Pregnancy on account of HDN, Plasma exchange, Platelet apheresis, ABO incompatible Transplantations etc.

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