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Prevalence of Anti-A and Anti-B Haemolysins in Group O Blood Donors at the National Blood Transfusion Center of Abidjan, Côte d'Ivoire

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Abstract: ABO blood-group system is characterized by the constant presence in the plasma of natural anti-A and anti-B antibodies (regular, agglutinating, class IgM) corresponding to the antigens absent on the membrane of the red blood cell. In addition to these natural antibodies, there may be immune antibodies (called haemolysins) in response to different types of immunological stimuli. These anti-A and anti-B haemolysins are capable of triggering the complete cascade of complement leading to haemolytic accidents in the case of non-isogroup ABO transfusion. Our objective was to determine the frequency of IgG anti-A, anti-B and anti-A + B haemolysins in group O blood donors at the national blood transfusion center of Abidjan. Our retrospective cross-sectional study was conducted at the national blood transfusion center of Abidjan and that investigated anti-A, anti-B and anti-A + B haemolysins in the sera of 191 group O blood donnors, aged 18-65 years, using the heat technique with direct agglutination of the serum to be tested by red blood cells A1 and B papained. The prevalence of IgG haemolysins was 35.08% (10.47% anti-A, 15.71% anti-B and 8.9% anti-A + B). The average age of our population was 32,9 years [18-63 years] with a male / female sex ratio of 4.97. Haemolysin levels were higher in men (27.75%) than in women (7.33%) (not a significant difference, p= 0.44). Age distribution showed a high haemolysin level in the 25-29 age group (significant difference, p = 0.001), with variable rate ranging from 2.1% to 6.3%. Ideally, group O blood should only be transfused to group O subjects, except in emergency situations where iso-group blood is not available. Since the technique used in our study to investigated haemolysins was a qualitative method, we could not have the title of the various haemolysins in order to assess the real risk of an immunological accident after such a blood transfusion.

Keywords: Haemolysins, Blood Donors, Group O, Côte d'Ivoire

1. Introduction

ABO blood-group system is characterized by the constant presence in the plasma of any individual of the natural antibodies anti-A and anti-B corresponding to the antigens absent on the membrane of the red blood cell. These antibodies are of class IgM, agglutinating with a thermal optimum at 4°C. In the ABO system, it is recommended to transfuse to "isogroup" (principle of pheno-identity) [1]. Otherwise, for reasons of shortage or need for emergency transfusions, non-isogroup transfusions (principle of pheno-identity) [1] are used. The ABO natural antibodies of the donor are safe for the recipient's red blood cells because they are rapidly diluted in the recipient's circulation and absorbed on the tissue antigens A and B of the vascular wall [2]. In addition to these natural antibodies, there may be immune antibodies. Indeed, following different types of immunological stimuli: 1. allogeneic stimulation by red cell antigens (incompatible ABO transfusion, pregnancy with incompatible ABO fetus, organ or tissue transplant from an incompatible ABO donor) [1, 3] or 2. heterogeneous stimulation (vaccination - tetanus toxoid, typhoid vaccine - serotherapy - tetanus serum, horse serum - and certain pharmaceutical preparations containing blood group substances, etc.), some individuals may develop ABO immune antibodies, irregular, especially of class IgG and possessing hemolytic activity at 37°C. These antibodies, called anti-A and anti-B haemolysins, are capable of triggering the complete complement cascade leading to hemolytic accidents in the case of non-isogroup compatible ABO transfusion. The search for these haemolysins in blood donors therefore falls within the framework of the immunological safety of transfusion. Indeed, the presence of anti-A and anti-B haemolysins in a certain number of subjects of group O has already been demonstrated in Africa as well as in Europe [4-8] making the subject O with haemolysins the "dangerous universal donor". Injection of such blood to recipients A, B or AB may cause severe haemolytic accidents. This type of blood O is therefore reserved for group O recipients (the presence of haemolysins is reported on the blood bags). Detection of natural antibodies is carried out by the Simonin serum test. That of immune antibodies can be made after neutralizing natural antibodies either by indirect Coombs technique using soluble group substances to absorb natural antibodies, by heat destruction, or by direct search for haemolysing effect of these antibodies [2]. The objective of our study was to determine the frequency of anti-A and anti-B haemolysins of anti-A, anti-B and anti-A + B haemolysins in group O blood donors at the national blood transfusion center of Abidjan.

2. Materials and Methods

2.1. Materials

Our study is part of the safety of transfusion. It was carried out at the national blood transfusion center in Abidjan, the economic capital of Côte d'Ivoire, situated in the southeast. This was a retrospective cross-sectional study that investigated anti-A and anti-B haemolysins in sera from 191 group O voluntary blood donors aged 18-65 years in 2012 (January to December) and whose records were properly filled out. The variables studied were sex, age and outcome of the search for haemolysins.

2.2. Methods

2.2.1. Equipments and Reagents

- Serum to be tested
- Non-papained red blood cells (RBC) A1 Rhesus negative and non-papained RBC B Rhesus negative
- Lyophilized papain stored at 4°C
- Papained RBC A1 Rhesus negative and papained RBC B Rhesus negative, put in salt suspension in 5%
- Hemolysis tubes, racks for hemolysis tubes, physiological water, pasteur pipettes, beakers
- Water-bath at 37°C and at 70°C

NB: The use of RBC A1 phenotype is imperative because the anti-A antibody always has anti-A1 specificity. RBC A1 and B should be Rhesus negative to avoid interference with a potential anti-D antibody.

2.2.2. Technique

This method was based on direct agglutination of neutralized serum in presence of papain treated RBC A1 and B.

a. Neutralization of natural antibodies anti-A and anti-B
   - Dilute the serum to be studied to 1/3 in physiological water.
   - Incubate in water bath for 15-20 minutes at 70°C. After 10 minutes, monitor the tube in search of an opalescent appearance.
   - Verify the neutralization of the natural antibodies by carrying out a Simonin test with RBC-test A1 and RBC-test B at 10%.
   - If the reaction is positive, incubate in water-bath for 10 min at 70°C.

b. Preparation of papained RBC A1 and B
   - In a hemolysis tube, put a volume of RBC A1 washed three times in physiological water.
   - Add the same volume of papain.
   - Incubate for 7 min at 37°C.
   - Wash three times at 3000 rpm for 2 minutes.
   - Make a 5% suspension in physiological water.
   - Repeat the same procedure with the RBC B.

c. Detection of immune anti-A and anti-B antibodies (anti-A and anti-B haemolysins)
   - In a haemolysis tube, put a drop of neutralized serum. Add with a drop of washed papained RBC A1.
   - In another hemolysis tube, put a drop of neutralized serum. Add a drop of washed papained RBC B.
   - Incubate in water-bath for 45 mn at 37°C.
   - Centrifuge at 1000 rpm for 1 min or at 3000 rpm for 20 sec.

2.2.3. Reading and Interpretation of Results

- Read the presence of agglutination by slightly waving the tube over a Kahn mirror or by observing it in front of a light source.
- Agglutination shows the presence of haemolysin in the serum studied.
- Absence of agglutination reflects the absence of haemolysin in the serum studied.

2.3. Data Analysis

The data were collected on a survey sheet from the computer system of the national blood transfusion center of Côte d'Ivoire. The data were entered, processed and analyzed using World, Excel software. For the statistical analysis, we used the chi-square test and Fisher's exact test with $\alpha = 5\%$ as the significance level.

3. Results

3.1. Overall Results

a. *Sex*: Out of the 191 donors, 159 (83.25%) were male and 32 (16.75%) were female, with a gender ratio of 4.97.

b. *Age*: The average age was 32.9 years with extremes of 18 years and 63 years. The 25-29 year old were the majority (26.7%) followed by the 30-34 year old (17.28%).

c. *Frequency of haemolysins IgG*

Out of the 191 sera studied, 67 or 35.08% showed agglutination in one or both tubes. This means the presence of one or both haemolysins.

3.2. Analytical Results

a. IgG haemolysins levels by sex

Table 1. *Prevalence of IgG hemolysins among the population studied by sex.*

	Haemolysins n (%)			Total n (%)
	Anti-A	Anti-B	Anti-A + Anti-B	
Male	17 (8.9)	24 (12.57)	12 (6.28)	53 (27.75)
Female	3 (1.57)	6 (3.14)	5 (2.62)	14 (7.33)
Total	20 (10.47)	30 (15.71)	17 (8.9)	67 (35.08)

Khi^2=2.67 ddl=3 p=0.44

Haemolysin levels were higher in men (27.75%) than in women (7.33%).

b. IgG haemolysin levels by age group

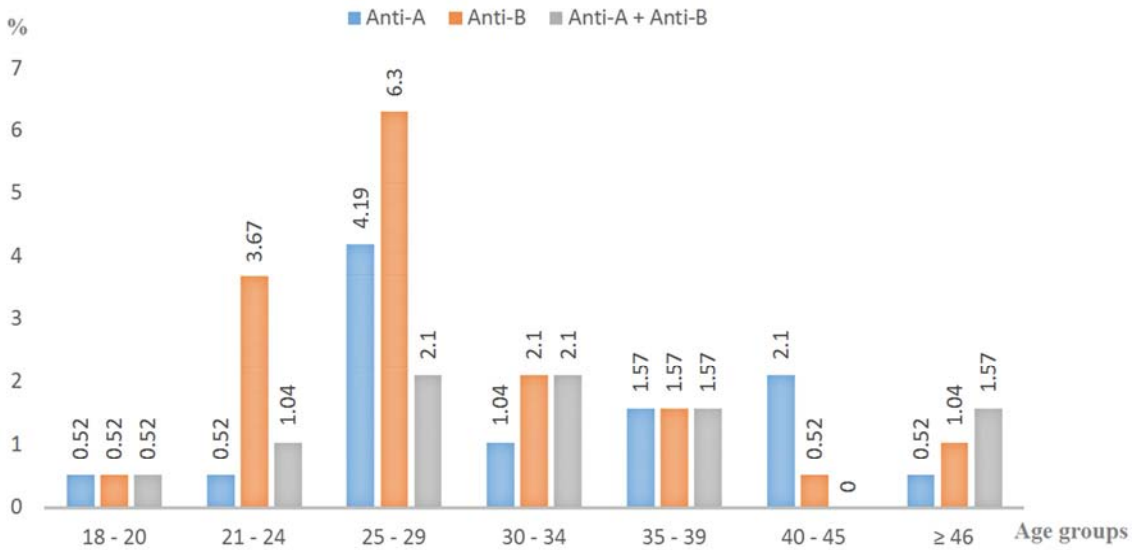

Figure 1. *Prevalence of IgG haemolysins among the population studied by age group Fisher's exact test: p=0,001.*

According to age, levels of anti-A, anti-B and anti-A + B haemolysins ranged from 0.52% to 6.3% with higher frequencies in the 25-29 age group (2.1% to 6.3%).

4. Discussion

4.1. Global Data

The search for haemolysins in blood donors is part of the immunological safety of transfusion. The injection of such a blood can cause, in case of non-isogroup compatible transfusion (blood O to patient A or B or AB, blood A to patient AB, blood B to patient AB), severe haemolytic recipient [2, 9]. This is particularly true when transfusing whole blood or blood products such as plasma or platelets. Today, in red blood cell concentrates, the amount of residual plasma is very low (≤ 25 ml). The risk is therefore limited

with this type of product, but the search for haemolysins remains mandatory [2]. The clinical value of haemolysins also lies in their ability to cause hemolytic disease of the newborn in the event of ABO fetal-maternal incompatibility. These haemolysins can cross the placenta and cause lysis of fetal blood of red blood cells [10].

Our study population consisted of 191 group O blood donors with 159 or 83.25% male donors versus 32, or 16.75% female; A gender ratio of 4.97 (about 5 men for a woman). The low proportion of female donors could be explained by blood donation criteria excluding pregnant women, lactating women and menstruating women, but also by the relatively low rate of women in high schools and universities (main places for mobile collection of labile blood products). The result obtained from this study revealed an overall prevalence of haemolysins of 35.08% in group O donors. Anti-A haemolysin occurred less frequently compared with anti-B haemolysin. This relatively high overall haemolysin prevalence is comparable to the reports of several authors from Nigeria.

- Olawuni [6]: In a prospective study of sera from 250 group O blood donors in 2001 in Ilorin, the prevalence of alpha and beta haemolysins was 23.2%. Anti-B haemolysins occurred twice as frequent as anti A haemolysins. There was no relationship between sex and age and the prevalence of haemolysins. Significant titre of 8 and above was found in 18.5% of anti-A and 13.2% of anti-B.
- Kagu [7]: In a prospective study over 2 years of 1929 voluntary group O blood donors (1609 males and 320 females, median age 26 years ±7.6 SD) in 2011 in northeastern, the overall prevalence of haemolysins was high: 55.4% (10.3% anti-A, anti-B 12.6% and 32.5% anti-A and anti-B). But the proportion of the whole study population with significant titre of 8 and above was low: 2% for anti-A and 2.8% for anti-B.
- Ugah [11]: Out of a total of 157 group O donors samples in 2014 in Federal Teaching Hospital, Abakaliki, 83 (52.87%) had haemolysins [33 (39.76%) anti-A, 26 (31.33%) anti-B and 24 (28.91%) anti-A and anti-B]. 52 (62.65%) had significant titre of 8 and above.
- Oyedeji [8]: in 2015, in a group of 350 voluntary group O blood donors in Lagos with age range 18-58 years and median age of 28 ± 8.4 years, 106 (30.3%) had haemolysins. The prevalence of anti-A haemolysins (15.4%) was significantly higher than that of anti-B haemolysins (5.1%). The prevalence of anti-A and anti-B haemolysins was 9.7%. A visual titer of 8 and above which is considered significant was seen in 18.6% of donor samples.

On the other hand, it is higher than that observed by Louati and Uko.

- Louati [2], in a study carried out in Tunisia in 2008 in 2824 blood donors, 132 (4.67%) had haemolysins. The ABO blood group study showed the following levels: 6.62% in group O donors (2.81% anti-A, 1.83% anti-B and 1.98% anti-A and anti-B); 2.08% in group A, and 5.04% in group B with a significant difference between the rates found in O and non O group donors. The difference between this study and ours could be explained by the small size of our sample.
- Uko [3] In a study conducted in northwestern Nigeria out of 140 donors (60 group O, 40 group A and 40 group B) found a prevalence of haemolysin of 10.0% (50% anti-A 28.5% Anti-B and 21.4% anti-A and anti-B) with a significant difference in group O donors (18.3%) compared to group A (5%) and group B (2.5%) donors.

In Côte d'Ivoire, there is a policy of donor loyalty in which a mail is sent to the donor thanking him for his donation and inviting him to return within one to two weeks for his results when there is an anomaly. Screening for haemolysins is recommended but not mandatory. However, it is routinely performed in all blood donors at the national blood transfusion center in Côte d'Ivoire. Moreover, the presence of hemolysins is reported on the blood bags and this type of blood O is reserved for group O recipients.

4.2. Analytical Data by Sex and Age Groups

In our series, the study by sex showed a hemolysin level of 27.75% in men and 7.33% in women with a non-significant difference ($p = 0.44$). The study by age group showed a high rate of hemolysins in the 25-29 age group, with variable rates ranging from 2.1% to 6.3%. This significant difference ($p = 0.001$) could be explained by the fact that most donors fall within this age range. There is little data in the literature comparing hemolysin levels by sex and age in group O. However, Louati [2] found significant differences between male and female levels in each age group of its series on the different groups of the ABO system.

5. Conclusion

Ideally, blood group O should only be transfused to group O subjects, except in emergency situations where there is no iso-group blood. Because some group O donors have been shown to possess immune ABO antibodies in their plasma, which are harmful to the recipient's red blood cells. These are anti-A and anti-B haemolysins encountered after allogeneic or heterogenic stimulation by red blood cell antigens. Our study aimed at assessing the prevalence of hemolysins showed a high incidence of hemolysins in group O donors in Côte d'Ivoire (35.08%). The study by sex and age showed that there was no statistically significant difference between these parameters and the presence of haemolysins. Since the technique used was a qualitative method, we could not have the titles of the various hemolysins in order to assess the real risk of an immunological accident after such a blood transfusion.

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