Research Article

Anti-A and Anti-B Haemolysins amongst Group “O” Voluntary Blood Donors in Northeastern Nigeria

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Background and Objective. The aim of this study was to determine the prevalence and haemolytic significance of alpha- and beta-haemolysins in our voluntary group “O” donor population. **Methods.** This was a prospective study carried out at North-East Zonal Centre, the National Blood Transfusion Service, Maiduguri, Nigeria from April 2007 to April 2009. One thousand nine hundred and twenty nine voluntary group “O” blood donors (1609 males and 320 females, median age 26 years $\pm$ 7.6 SD) were screened for alpha- (anti-A) and beta- (anti-B) haemolysins using the standard tube technique at 37 degrees C for 1 hour. All samples showing haemolysis were titrated for anti-A and anti-B haemolysins. **Results.** The overall prevalence of haemolysins in group O donors was 55.4%. Prevalence of alpha- and beta-haemolysins only was 10.3% and 12.6%, respectively, while that of donors having both alpha- and beta-haemolysins in their sera was 32.5%. Visual titre of 8 was seen in 0.4% of lytic alpha-haemolysin and 0.2% of lytic beta-haemolysin whereas donors with both alpha- and beta-haemolysins had a titre of 1.8%. Lytic titre of 16 and 32 was very low in our donor population. **Conclusion.** This study has shown that although the prevalence of haemolysins is high in our voluntary group “O” donor population, the strength of the lytic antibodies is low. Therefore, despite the labour intensiveness of our haemolysis titration technique and the frequent transfusion of group O blood to certain recipients of blood group A, B, and AB in our environments, there is the need to routinely screen our donors for haemolysins in order to identify those posing the greatest risk to recipients. Further studies to determine episodes of clinically significant haemolysis in recipients of blood group O may be necessary.

1. Introduction

The importance of a blood group system in clinical blood transfusion practice lies in the frequency of its antibodies and in the possibility that such antibodies will destroy incompatible cells in vivo [1]. Almost everybody over the age of 6 months has clinically significant anti-A and/or anti-B in their serum if they lack the corresponding antigens on their red cells [1]. Blood group “O” red cells can be given to A, B, or AB recipients and were formerly inappropriately called “Universal donor red cells”. Early studies from Nigeria have shown high frequency of potentially lytic anti-A and lytic anti-B in blood group “O” persons [2, 3]. These high

frequency of alpha- and beta-haemolysins has been suggested to be responsible for the high frequency of ABO-haemolytic disease of the newborn seen in Africans [4, 5]. Blood group “O” is the commonest and most prescribed blood group type in our environment [6]. The fractionation of blood into its various components is still a nightmare in Nigerian blood transfusion service centres and the hospital blood banks. Apathy to blood donation coupled with poor funding is mostly responsible for these difficulties. As such most clinicians often transfused group compatible whole blood to recipients who ordinarily need packed red cells and/or fresh plasma. The occurrence of anti-A (alpha-haemolysin) and anti-B (beta-haemolysin) in group “O” donors was reported

TABLE 1: Distribution pattern of haemolysins among the group “O” donors.

Gender	Anti-A	Anti-B	Anti-A+B	Negative for haemolysins	Total (%)
Male	159 (8.2)	220 (11.4)	528 (27.4)	702 (36.4)	1609 (83.4)
Female	40 (2.1)	24 (1.2)	98 (5.1)	158 (8.2)	320 (16.6)
Total	199 (10.3)	244 (12.6)	626 (32.5)	860 (44.6)	1929 (100)

TABLE 2: Visual titres of anti-A, anti-B, and anti-A+B among the group “O” donors.

Titre	Anti-A	Anti-B	Anti- A+B	Total (%)
1	33 (3.0)	34 (3.2)	87 (8.1)	154 (14.3)
2	106 (9.9)	127 (11.9)	336 (31.4)	569 (53.2)
4	53 (5.0)	78 (7.3)	179 (16.7)	310 (29.0)
8	4 (0.4)	2 (0.2)	19 (1.8)	25 (2.4)
16	2 (0.2)	1 (0.1)	4 (0.4)	7 (0.7)
32	1 (0.1)	2 (0.2)	1 (0.1)	4 (0.4)
Total	199	244	626	1069 (100.0)

to be high in African population [7–9], and as such, some laboratories still spend a great deal of time screening for these lytic haemolysins using the labour-intensive technique due to the nonavailability of automated technique. The prevalence of these haemolysins also varies with age and gender [8, 9].

No similar study was carried out in this part of the country to investigate the prevalence and the strength of alpha- and beta-haemolysins in our voluntary group “O” donor population, hence the necessity of this study.

2. Materials and Methods

2.1. Materials. Blood samples for the study were obtained from voluntary group “O” donors, who had been screened, found fit, and accepted as donors. About 4 mls of haemoglobin-free serum was obtained from clotted samples, and these were stored at minus 18–20°C until they were analyzed (all samples were tested within 12 hours of separation after addition of absorbed fresh O serum as a source of complement). Although the lytic property of serum deteriorates rapidly on storage due to decay of complements, storage is to avoid the effect of high temperature in our environment that would also affect the potency of antibodies [10].

2.2. Methods. One volume of donor serum and one volume of absorbed fresh O serum (as a source of complement because the lytic property of serum deteriorates rapidly on storage due to decay of complements) were placed into each of 3 test tubes. To each tube was added 1 volume of 5% suspension in saline solution of red cells of group A, B, and O, respectively. The O cells were used as negative control. The tubes were then incubated at 37°C for 1 hour, after which all tubes were centrifuged. They were then held before a source of light, and with minimal disturbances, the supernatant was examined for haemolysis microscopically. Haemolysis

was graded as follows: 3+: complete haemolysis, 2+: partial (more than 50% but not complete) haemolysis, 1+: trace haemolysis, and negative, no visual visible haemolysis. All samples showing haemolysis were titrated for anti-A and anti-B haemolysins as follows: 2 mls of each serum was double diluted serially in saline up to 256 and 0.5 mls of each serum dilution and 0.5 mls of absorbed fresh group O serum were placed in each of 3 tubes. To each tube was then added 0.5 mls of 5% A-cells, B-cells, and O-cells, respectively and the mixture was incubated at 37°C. At 1 hour, the samples were examined for haemolysis microscopically, and the titres were recorded. Titres were recorded as the dilution showing the weakest haemolysis microscopically. All data were analysed by standard statistical software (SPSS, Chicago, IL, USA). A *P* value of <.05 was considered significant.

3. Results

One thousand nine hundred and twenty nine sera from voluntary group “O” donors (1855-Rhesus D Positive and 74 Rhesus D Negative) were examined by the method described for anti-A and anti-B haemolysins. The median age of the donors was 26 years +7.6 SD. These included 1609 males and 320 females. The overall prevalence of haemolysins in group O donors was 55.4%. Alpha- and beta-haemolysins only was seen in 10.3% and 12.6% of the donors, respectively, while that of donors having both alpha- and beta-haemolysins in their sera was 32.5% (Table 1). Visual titre of 8 was seen in 0.4% of lytic alpha-haemolysin and 0.2% of lytic beta-haemolysin whereas donors with both alpha- and beta-haemolysins had a titre of 1.8% (Table 2). Lytic titre of 16 and 32 was very low (Table 2). There was no statistically significant difference between male and female donors in the frequency of haemolysins. Age also has no significant impact on the frequency of haemolysins in this study.

4. Discussion

This study has confirmed the high frequency of haemolysins in Nigerian group “O” donors. The prevalence of 55.4% observed in this study is higher than those reported by Olawumi and Olatunji in Ilorin (Southwestern Nigeria) [8] in 2001, Worlledge et al. (Southsouthern Nigeria) [3] in 1974, and Kulkarni et al. in Zaria (Northwestern Nigeria) [2] in 1985 as well as the work reported by Onwukeme and Nanna in Jos (North-central Nigeria) [11] in 1990. The higher prevalence rate observed in this study could be

due to the methods and the fact that large population of group "O" donors were screened. Another possible reason could be the geographical location of Maiduguri bordering the Republic of Cameroun to the south, the Republic of Niger to the North, and the Republic of Tchad to the east. Admixture of blood of immigrants as a result of intermarriages could also be responsible for the higher prevalence. This geographical variation in the prevalence has been described in the literature [2, 11]. There was no statistically significant difference between male and female donors in the frequency of haemolysins. This is in conformity with the work reported by other workers [8, 10]. There was also no significant age difference in the prevalence of haemolysins. This is consistent with the findings of other workers [8, 12]. Although episodes of haemolysis in the recipients were not determined in this study so as to justify the clinical significance of such antibodies, it will be an overstatement to state that the frequency of strongly lytic anti-A and anti-B is low in this study. Taking a visual titre of 8 and above as being able to cause significant in vivo haemolysis [13], only 0.4% of the sera positive for alpha-haemolysin and 0.2% of those positive for beta-haemolysin had significant visual titre of 8. Similarly, a titre of 16 was seen in only 0.2% for haemolytic anti-A and 0.1% for haemolytic anti-B. This finding is lower than that reported by Olawumi and Olatunji [8] who found a significant prevalence titre of 2.0% for anti-A and 2.8% for anti-B. The difference could be due to the methods and the large sample size used in this study. In a study of the distribution of anti-A haemolysins in group "O" donors, David-West [14] found most titres to be not more than 16. In this study, most titres are between 2 and 4. This study has shown that although the prevalence of haemolysins is high in our voluntary group "O" donor population, the strength of the lytic antibodies is low. Therefore, despite the labour intensiveness of our haemolysis titration technique and the frequent transfusion of group O blood to certain recipients of blood group A, B, and AB in our environments, there is the need to routinely screen our donors for haemolysins in order to identify those posing the greatest risk to recipients. A further study to determine the episodes of haemolysis in the recipients of blood group O is needed so as to justify the clinical significance of such antibodies.

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