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Prevalence and titre of alpha and beta haemolysins in blood group 'O' donors in Ilorin

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Summary
Sera from 250 selected group 'O' donors were screened for anti A and anti B haemolysins. Those that were positive were titrated for haemolytic antibodies and the result read both visually and spectrophotometrically. The visual and spectrophotometric titres were then compared. The prevalence of α and β haemolysin was 23.2%. Haemolytic anti B occurred twice as frequent as haemolytic anti A but the titre of haemolytic anti-A was higher than haemolytic anti-B. There was no relationship between sex and age and the prevalence of haemolysins. Significant visual titre of 8 and above was found in 18.5% of lytic anti-A and 13.2% of lytic anti-B, but the proportion of the whole study population with significant visual titre of 8 and above was low, i.e., 2.0% for anti-A and 2.8% for anti-B. On the average the spectrophotometric titres were consistently one fold higher than the visual titres.

Keywords: *Group 'O' donors, haemolysin, prevalence*

Résumé
Séra prélèvé de 250 donnateurs groupe 'O' sont examiné pour anti A et anti B hémolysines. Ceux qui avaient montré positif étaient titré pour determiner les anticorps hémolytique et le résultat était lu visuellement et avec la spectrophotometre à la fois. Les "titres visuel et de spectrophotomètre s'étaient alors comparés. La fréquence d'hémolysin α et β était 23,2%. L'hémolytique anti B se produit deux fois si fréquente que l'hémolytique anti-A mais le "titre" d'hémolytique anti-A était plus élevé que l'hémolytique anti-B. Il n' y avait pas un rapport entre le sexe l'âge et la fréquence d'hémolysin. Le titre visuel signifiant de 8 et plus était trouvé dans 18,5% de lytique anti-A et 13,2% de lytiqui anti-B. Mais la proportion de l'entier de la population étudiée ayant un titre visuel signifiant de 8 et plus était basse c-à-d 2,0% pour anti-A et 2,8% pour anti-B. Au moyen, les titres spectrophotomètriques sont invariablement plus élévé que les titres visuels.

Introduction

Ideally, group 'O' blood should be transfused only to group 'O' recipients, except in emergency situations when group 'O' donor blood is used as universal donor where group identical blood is not available. This is particularly the practice in blood banks with inadequate blood supply, and in the transfusion of infants born to non-group identical mothers [1]. This universal donor phenomenon is outdated in major blood banks especially in developed countries because it has long been recognized that certain donors possess in their plasma potent ABO antibodies, which are dangerous to the recipients' red cells. These are anti-A and anti-B haemolysins found after allogenic stimulation by red cell antigens. Sources of stimuli include allogenic stimuli from ABO incompatible blood donation, pregnancy with an ABO incompatible fetus and organ transplantation from a non ABO-matched donor [2,3]. Others are heterogenic antigens in the form of A and B substances found in tetanus toxoid, antitetanus serum (horse serum) and typhoid A, B (TAB) vaccine [4].

The second clinical significance of anti-A and anti-B haemolysins is their ability to cause haemolytic disease of the new born (HDN) due to ABO incompatible pregnancy. The incidence and severity of haemolytic disease of the newborn are significantly greater in Africans than Caucasians [5,6]. It has been suggested that the greater incidence of HDN in Africans is due to the very high haemolytic activity of anti-A and anti-B in black group 'O' individuals [7,8].

The higher prevalence of anti-A and anti-B haemolysins in Africans has been alluded by Worlledge to be due to blood sucking insects. [7].

The prevalence of alpha and beta haemolysins ranges from one part of Nigeria to the other. Reports have been in the range of 30 – 56% [1,8,9]. Haemolysin titres are usually in the range of 2 to 32 but a visual titre of 8 has been observed to be potent enough to cause in-vivo haemolysis [1,10]. The haemolysin screening test is performed routinely in some blood banks to identify "dangerous" group 'O' blood containing the potent haemolysin in the donor pool [11]. However some other hospital blood banks including University of Ilorin Teaching Hospital do not carry out the same screening inspite of the reported high prevalence of haemolysins among group 'O' donors.

No work on the prevalence of haemolysin among group 'O' donors has been done in this part of the country. This work is aimed at determining the prevalence and titre of alpha and beta haemolysins in group 'O' donors in Ilorin. It will also compare the visual titres with titres by spectrophotometric readings so that recommendations can be made as to the necessity or otherwise of routine screening as well as the usefulness of spectrophotometry in haemolysin titration.

Materials and methods

Materials

Blood samples for the study were procured from 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 analysed.

Methods

One volume of donor serum and one volume of absorbed fresh O serum (as a source of complement) were placed into each of 3 test tubes. To each tube was added one volume of 5% suspension in saline solution of red cells of groups 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 time all tubes were centrifuged. They were then held before a source of light and with minimal disturbances the supernatant was examined microscopically for haemolysis. Haemolysis was graded as

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follows: 3+, complete haemolysis, 2+, partial (more than 50% but not complete) haemolysis, 1+, trace haemolysis, negative, no visual haemolysis. All samples showing haemolysis were titrated for anti-A and anti-B haemolysins. 2 mls of each serum was double diluted serially in saline up to 256, and 0.5 mls of each serum dilution and 0.5ml of absorbed fresh group O serum were placed in each of 3 tubes.

To each tube was added 0.5ml of 5%, A cells, B cells and O cells respectively. The O cells were used as a negative control and the tube that contained it served as serum blank tube. All tubes were incubated at 37°C for 1 hour. At the end of incubation they were centrifuged and the supernatant examined for haemolysis visually. The visual titre was taken as the last dilution of serum where haemolysis was seen. After visual assessment of haemolysis, other tubes were set up in identical arrangement to the incubated ones and 3.2ml of saline was placed in each of them. About 0.8mls of supernatant was carefully pipetted from each centrifuged tube into the corresponding tube in the second set with thorough mixing to produce a 1 in 5 dilution of each suspension. The optical density of each was read on a spectrophotometer at 540 nm using saline as blank. The titre was taken as the dilution just before that which gave the same optical density as the serum blank.

Results

Two hundred and fifty sera from group 'O' donors within the age range 18–59 years selected at random were examined by the method described for anti-A and anti-B haemolysins. These included 239 males and 11 females. Of the total donor population 58 (23.2%) had alpha and/or Beta haemolysin. Haemolytic anti-A was found in 27 (10.8%), anti-B was found in 53 (21.2%) (Table I).

Table 1: Visual and spectrophotometric titres of Anti A and Anti B among group O donors

Titres	Number of Donors			
	Anti A		Anti B	
	Visual	Spectrophotometric	Visual	Spectrophotometric
2	0	4	0	
7	4	15	7	
13	6	27	13	
4	13	6	27	
0	3	1	5	
1	0	0	1	
0	1	0	0	
22	27	53	53	

However, antibody titres were higher for anti-A than The minimum titre for both anti A and anti B was 1 maximum titres were 16 and 32 for anti A and anti B, ely. Of the 58 donors with haemolysins, only 10 had t titre (i.e. visual titre of 8 and above). Twenty-two 8%) had both anti-A and anti-B haemolysins, 5 do- and 31 donors (12.4%) had only anti-A and anti-B n, respectively. No serum containing anti A only nificant titre whereas 5 were significant when in com- ith anti B. On the other hand 4 sera with Anti B only had significant titre (See Table 2S). There was ant influence of age or sex on the frequency of s.

When the visual titres were compared to those of th spectrophotometer only 8 (10%) out of 80 titrations gave th same titres both visually and spectrophotometrically. In th remaining 72 (90%) titrations, spectrophotometric titres wer one tube higher than the visual titres. So on the average ther was a one-tube difference between the visual titre and that of th spectrophotometer.

Table 2: Pattern of distribution of haemolysins

	No. positive for Haemolysin	%	No. with haemolysin titre of 8	≥	%
Anti A only	5	2.0		0	0
Anti B only	31	12.5		4	1
Anti A + B	22	8.8	Anti A only	3	1.
			Anti B only	1	0.
			Anti A and Anti B	2	0.
Sub total	58	25.3		10	
No haemolysin	192	76.8		0	
Total	150	100		10	

Discussion

A policy of transfusing only group identical donor blood recipients would make the screening of blood for haemolysi unnecessary. However, the non vailability of donor blood of groups at all times necessitates the transfusion of group 'O donor blood to certain recipients of A, B and AB blood grou particularly in developing countries. Unfortunately not all th blood banks that give group compatible rather than group iden tical blood engage in screening for haemolysin including U.I.T.H hence the necessity of the present study.

This study showed a haemolysin prevalence of 23.2 in the 250 blood group 'O' donors. This is slightly lower com pared to those reported by Onwukeme in Jos (38.1%) in 19 [9] and Kulkarni et al in Zaria (32.1%) in 1985 [8]. David-We [1] and Worlledge *et al.* [7] had previously reported a muc higher prevalence of 56% and 85%, respectively. In the latt report the extremely high prevalence could be attributed to th method of testing in which 1% suspension of cells in saline wa used because it has been reported that the higher the serum co ratio the higher the susceptibility to red cell lysis [12].

The lower haemolysin prevalence in the present stud could be due to the fact that Ilorin is located between the Nort and South, with admixture of blood of immigrants from th Savannah North as a result of intermarriages between them This is consistent with the finding of Kulkarni *et al* [8] an Onwukeme [9] of variations in haemolysin prevalence in th different ethnic groups, Hausa of the Savannah North (20% Igbo (40%) and Yoruba of the forest region of Nigeria (50 60%). There was no positive difference between males an females in the frequency of haemolysin. This is in the keepin with the work done by Adewuyi *et al* among black Zimbabwe ans [13]. There was also no significant age difference in th prevalence of haemolysin. This is consistent with the finding o Sapphire *et al* in 1993 [14].

Alpha haemolysin occurred less frequently compare with beta haemolysin, but haemolysin titres were higher fo anti-A than anti-B. This is also consistent with the findings o Adewuyi *et al* among black Zimbabweans [13].

Taking a visual titre of 8 and above as being able t cause significant in-vivo haemolysis [1], 18.5% of the sera posi tive for haemolytic anti-A and 13.2% of those positive fo haemolytic anti-B had significant titres. The percentage of th total study population with significant titres was just 2.0% fo

anti-A and 2.8% for anti-B. This is much lower than those found in studies in Black group 'O' donors of Zimbabwe (18.6%) [13]). However in a study of distribution of anti-A haemolysins in group 'O' donors, Polley *et al* [14] found a bimodal pattern of haemolysin titres with one peak at a very low titre of 1 and the other 8. Since sera that produced a moderate grade (2+) haemolysis had titres in the range of 4-8, it can be said that any sera that produces (2+) haemolysis should be considered as having significant lytic antibodies. And of course those with 3+ haemolysis are dangerous for transfusion to a non-group 'O' recipient. Those with slight (1+) haemolysis may be considered as not having significant lytic antibodies but as much as possible they should not be transfused to non-group 'O' recipients. If it has to be used, it should be as packed cells. Of importance in this study is the finding that alpha haemolysin, when it exists alone did not attain significant titre while significant titre is attained when beta haemolysin exists alone. Alpha haemolysin in combination with beta haemolysin on the other hand attains significant titre. This suggests that beta haemolysin may have a potentiating effect on alpha haemolysin when they occur together in a donor.

When the visual titres were compared with the spectrophotometric titres it was found that 90% consistently had titres of one tube higher spectrophotometrically. So it can be concluded that the spectrophotometric titre that can cause significant in-vivo haemolysis is 16 and above. It can also be predicted that some of the sera that were screened negative could be positive if the spectrophotometer was used for the screening, but of course the titre would be low (about 1). So it would not be necessary to use spectrophotometer for screening routinely because a low titre such as 1 is not clinically significant.

This study shows that significant haemolysin frequency of our group 'O' donor population is low and routine screening may not be necessary. But when an elective transfusion of 'O' blood group to a non-'O' individual becomes necessary, the group 'O' blood should be screened for haemolysin. Above all, close monitoring of all transfusions may be all that is necessary to prevent significant clinical consequence of such transfusions.

References

1. David-West AS: Blood transfusion and blood bank management in a tropical country. Clinics in Haematology 1981; 10:1014 - 1028.
2. Zimmerlim, Lerut J, Miller P, Zimmerman A, Nydegger U: Fluctuation of anti-A and anti-B histo-blood-group antibodies in a patient after liver transplantation. In fusions therapies 1991; 18:91 - 95.
3. Bushs JP, Maillard H, Nydeggar U: Anti-A/B-igm/G immune response to accidental A-O transfusion and to toxocara canis infestation. Schweiz med Whochenschr 1991: 121: 347- 350.
4. Turgeon ML. Fundamentals of immunohaematology theory and technique. USA: Lea and Febinger: 1989: 110 – 121.
5. Kirkman NN: Further evidence for a racial difference in frequency of ABO haemolytic disease. J Pediat 1977; 90:717-721.
6. Chintui C, Zipursky A, Blajchman M: ABO haemolytic disease of the newborn. East Afr. Med J 1979; 56: 314-319.
7. Worlledge S, Ogiemudia SE, Thomas CO, Ikoku BN, Luzzato L: Blood group antigens and antibodies in Nigeria. Ann Trop Med Parasitol 1974; 68:249 – 264.
8. Kulkarni AG, Ibazebe R, Fleming AF: High frequency of anti-A and anti-B haemolysins in certain ethnic groups in Nigeria. Vox Sang 1985; 48:39-41.
9. Onwukeme KE, Nanna OU: Frequency of anti-A and anti-B haemolysins in Nigerians living in Jos. Nig Med pract 1990; 20:29.
10. Kiedan SE, Lohan E, Mainwaring D: Acute anuria in haemophiliac due to transfusion of incompatible plasma. Lancet 1966:1: 179.
11. Fleming AF: Transfusion service handbook for ABU teaching hospital – page 9.
12. Polley MJ, Adinolfi M, Mollison PL: Serological characteristics of anti-A related to type of antibody protein (7s or 19s (Vox Sang 1963; 8:385 – 409.
13. Adewuyi JO, Gwanzura C, Mvere D: Characteristics of anti-A and anti-B in Black Zimbabweans. Vox Sang 1994; 67: 307- 309.
14. Saphire DG, Rudolph NS, Hackleman SM, Stone WH: The effect of age on the level of human ABO blood group antibodies. Aging 1993; 5: 177 – 184.