ORIGINAL ARTICLE

Prevalence of anti-A and anti-B hemolysis among blood group O donors in Lagos

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Abstract

Background: Group O donor blood is more readily available and is frequently used as universal red cell donor in our environment. The presence of hemolysins in the donors may however lead to hemolysis in the recipients. Attempts have been made to study the prevalence of hemolysins in various populations with results from our environment showing wide variation (20–80%).

Aims: To determine the prevalence and titer of anti-A and anti B hemolysins among blood donors at the Lagos University Teaching Hospital and compare results with that obtained elsewhere. Determine if the practice of transfusion of group O blood to nongroup O recipients is permissible in this environment.

Materials and Methods: Test for hemolysis was done using the standard tube method. Samples positive for hemolysis were then scored and titrated with the titers read visually and photometrically at 540 nm.

Results: Three hundred and fifty blood group O donors with age range 18–58 years and median age of 28 ± 8.4 years were enrolled in the study. The overall prevalence of anti-A and/or anti-B hemolysins obtained was 30.3%. Prevalence of anti-A and anti-B hemolysins only was 15.4% and 5.1% respectively whereas both anti-A and anti-B hemolysins were present in 9.7% donor samples. Though anti-A hemolysins were more prevalent than anti-B hemolysins, anti-B hemolysins had higher mean visual (6:7) and spectrophotometric titers (81:101). A visual titer of 8 and above which is considered significant was seen in 18.6% of donor samples.

Conclusion: Anti-A and anti-B hemolysins exist in significant frequencies and titers among blood group O donors in Lagos. It is recommended that the use of group O donor blood for recipients who are non-O be discouraged. Clinical studies to determine the frequency and severity of hemolysis in non-group O recipients of blood group O are required.

Key words: Anti-A and anti-B hemolysins, blood donors, blood group O, prevalence

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Introduction

Group O donor blood is more readily available with over 52% of population being blood group O in Lagos[1] [Table 1]. The relative difficulty in getting ABO group identical blood sometimes lead to the transfusion of group O donor blood to patients who are nongroup O in emergency situations and in cases of neonates born to non-ABO group identical mothers based on the assumption that blood group O donors are "universal donors of red cells."

The value of universal red cell donor blood is the fact that its red cells possess no antigens that may be attacked by the naturally occurring ABO blood group antibodies of the recipient. The blood group O plasma, however contains naturally occurring IgM anti-A and anti-B antibodies which are incompatible with the red cells of A or B recipients. These, usually, do no damage because they are usually hemagglutinin which after agglutinating corresponding cells, such cells get rapidly dispersed in patient's plasma.[2]

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Table 1: ABO typing of blood donors at the Lagos University Teaching Hospital University Teaching Hospital (January–December 2008)

Month	O Pos	O Neg	A Pos	A Neg	B Pos	B Neg	AB Pos	AB Neg	Total
January	359	19	169	6	202	4	19	3	781
Febuary	351	21	137	9	113	6	21	6	664
March	369	32	166	11	140	12	32	0	762
April	514	25	228	14	195	9	26	3	1014
May	439	26	159	10	148	6	21	1	810
June	589	33	244	9	92	11	40	4	1022
July	412	27	167	6	151	10	19	1	793
August	396	28	165	8	150	11	24	3	785
September	333	19	132	9	121	9	25	0	648
October	261	12	113	0	94	4	22	1	507
November	317	23	168	8	143	4	14	3	680
December	339	18	153	7	128	8	18	1	672
Total	4679	283	2001	97	1677	94	281	26	9138
	54.30		22.96		19.38		3.36		

Emphasis in transfusion service is thus focused on reactions between the recipients' antibodies and the donor red cells and not the antibodies in the transfused blood.

However, it is known that some blood group O donors have immune (IgG) anti-A and anti-B antibodies. These antibodies are also known as hemolysins and if present in sufficient high titer cause hemolysis of red cells of recipients when blood from these donors is transfused to nongroup O recipients.[3] These immune anti-A and anti-B antibodies are believed to have been acquired through previous blood transfusion, pregnancy or through vaccination. Furthermore, mosquito bites and intestinal parasitic infections have been suggested as means of acquisition.[4] Because of their lower complement-activating reactivity, the immune anti-A and anti-B antibodies, usually, need to be present in a higher concentration than the corresponding naturally occurring antibodies to cause an intravascular hemolytic transfusion reaction (IHTR).[5]

Restriction of the volume of transfused ABO-incompatible plasma will lead to a reduction in IHTR from both hemagglutinins and hemolysins. This can be achieved by transfusion of blood components. However, component therapy is still not routinely practiced in our environment as the required equipment and infrastructure to prepare blood components are lacking. Patients who need red cell or fresh plasma transfusion invariably get transfused with whole blood in most cases. If the immune anti-A and anti-B antibody titer present in such donor blood is high, there is a higher probability of hemolysis in transfused nongroup O recipients.

The prevalence of hemolysins has been studied in various populations with a high frequency of strongly hemolytic anti-A and anti-B hemolysins reported from Asian and Black populations compared with Caucasians.[6,7]

Several studies in Nigeria suggest that the prevalence is high in our environment with rates varying from 23.2%[8] to as high as 85%.[9] Data on prevalence of these hemolysins in group O donors in our environment thus require to be reviewed due to the wide variation in the prevalence earlier quoted.

Materials and Methods

Subjects/specimen collection: Blood samples were collected from 350 group O persons who were screened and found fit to be accepted as blood donors. A volume of 10 ml of venous blood was collected into plain (uncoagulated) sample bottles from each donor. The samples were allowed to clot at room temperature, and hemoglobin-free serum was obtained by centrifugation and pipetting the supernatant into corresponding well-labeled cryotubes for storage at −80°C until analyzed.

Methods

The hemolytic properties of IgG anti-A and anti-B was adopted for this test.

A volume of donor serum was placed into each of three test tubes. 5% suspension in saline of known red cells of groups A, B and O respectively were prepared. The O cells served as negative control. An equal volume of the red cell suspension was added to each test tube containing the donor serum. All tubes were incubated at 37°C for 1 h. The supernatant for each tube was examined under a light source for hemolysis. Hemolysis found in the tubes was graded as follows: 3+ = complete hemolysis, 2+ = partial hemolysis >50% but not complete, 1+ = trace hemolysis, and negative = no visible hemolysis. In samples that showed hemolysis, anti-A and anti-B hemolysins were titrated for thus: 150 μl of donor serum was placed on the first row of a 12 × 8 well microplate corresponding with the cell which showed hemolysis and also for O cells. The samples were then double diluted serially in saline up to 256. 150 μl of 5% A or B and/O cells suspension

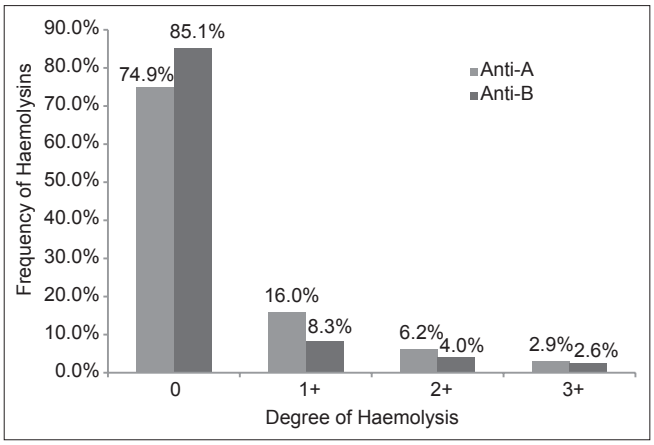

Figure 1: Frequency of hemolysins and the degree of hemolysis

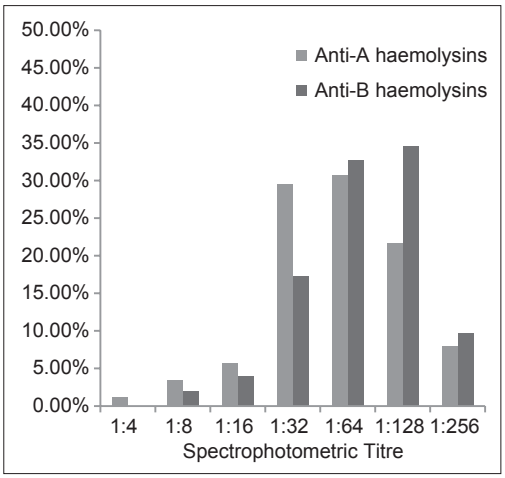

Figure 2: Visual and spectrophotometric titers and cumulative frequencies for anti-A and B hemolysins

was added to the wells, (the O cells served as a negative control and the well containing it served as serum blank well). All microplates were incubated at 37°C for 1 h. The supernatant of each well was visually examined for hemolysis with the visual titer taken as the last serum dilution where hemolysis is seen. Following visual assessment of hemolysis, other microplates were set up corresponding with the ones above. 120 µl of saline was placed into each well. 30 µl of the supernatant from the corresponding incubated wells above was pipetted into each of the new wells and then thoroughly mixed to produce a 1 in 5 dilution of each suspension. The optical density of each suspension was then read on a spectrophotometer at 540 nm using saline as blank. The spectrophotometric titer was taken as the dilution just before that which gives the same optical density as the serum blank.

All data were entered into a Microsoft excel spread sheet and analyzed using standard statistical software Epi Info version 3.3.2 developed by Centers for Disease Control and Prevention, Atlanta, Georgia USA. Comparison of mean values involved use of Student's *t*-test whereas discrete variables were compared using Chi-square test. $P < 0.05$ was considered as significant.

Results

Sera from 350 voluntary blood group O donors (328 Rhesus D Positive and 22 Rhesus D Negative) were screened for anti-A and/or anti-B hemolysins by the method described above. The donor ages were between 18 and 58 years with a median age of 28 ± 8.4 years. There were 331 males and 19 females.

One hundred and six (30.3%) donor samples had anti-A and/or anti-B hemolysins. Hemolytic anti-A and anti-B was found in 25.1% and 14.9% donor samples respectively (15.4% had anti-A only, 5.1% had anti-B only and 9.7% had both anti-A and anti-B hemolysins). The prevalence of anti-A hemolysins was significantly higher than that of anti-B hemolysins in the study population. The degrees of hemolysis and their frequencies are as illustrated in Figure 1.

Table 2: Mean, SD and median of visual and spectrophotometric titres of haemolysins A (*n*=88) and B (*n*=52)

	Visual		Spectro-photometric	
	Titer A	Titer B	Titer A	Titer B
Mean	6.3	7.3	80.9	101.2
SD	4.7	4.1	64.8	64.5
Median	4	8	64	64
Minimum	2	2	8	32
Maximum	32	16	256	256

SD=Standard deviation

Visual titers for anti-A hemolysins ranged from 1:2 to 1:32 while those for anti-B hemolysins ranged from 1:2 to 1:16. Spectrophotometric titers for anti-A and anti-B hemolysins ranged from 1:4 to 1:256 and 1:8 to 1:256 respectively [Figure 2]. Lower titers appear to be more common with anti-A than anti-B hemolysins both visually and spectrophotometrically although the differences are not significant. Anti-A and anti-B hemolysins with visual titer of 8 or higher was found in 10% and 8.6% of samples respectively.

The mean visual and spectrophotometric titers of anti-B were higher than those of anti-A but *t*-test and distribution did not show any significance in both the visual and spectrophotometric titers although there was a trend toward significance [Table 2]. Age, gender and rhesus blood group did not have any statistical significance on the frequency of hemolysins in this study population.

Discussion

Blood transfusion in our environment usually follows the rule of transfusing only group identical blood to a recipient after a major crossmatch. However, situations often arise when there is nonavailability of specific donor blood of all

groups, emergency situations where there is no time to wait for a crossmatch and in neonates born to non-ABO group identical mothers which necessitate transfusion of group O donor blood to recipients who are blood groups A, B and AB. Because group O individuals makeup over 52% of the population,[1] group O donor blood is often more readily available in our blood banks. Transfusion of group O blood to nongroup O recipients is performed with the impression that blood group O donors are universal donors of red cells based on the fact that blood group O cells possess no antigens, which the nongroup O recipient has antibodies against without taking into consideration the presence of hemolysins in such donor blood.

Routine screening for hemolysins is not performed in our blood bank but with a background information of high prevalence of hemolysins among blacks and from results obtained from other studies within Nigeria,[6,7,9-11] the knowledge of the prevalence in our environment may suggest the need for routine screening in preparation for those emergencies that require transfusion of group O donor blood to nongroup O recipients.

The prevalence of anti-A and/or anti-B hemolysins obtained in this study was 30.3%. This is comparable with those reported by Kulkarni *et al*. that found a prevalence of 32.3% in Zaria in 1985[10] but slightly lower than that reported by Onwukeme and Nanna that reported a prevalence of 38.1% in Jos in 1990.[12] However, the results of this study were lower than those reported by Worlledge *et al*. who had a prevalence of 85%,[9] David-West who reported a prevalence of 56%[13] and Okafor and Enebe who reported a prevalence of 53.6% for anti-A and 62.7% for anti-B hemolysins.[11] The result was higher than those reported by Olawumi and Olatunji, who found a prevalence of 23.2% in Ilorin in 2001.[8]

The differences in prevalence obtained by various studies in Nigeria may be attributed to the variation in the serum-cell ratio used in different studies. It has been reported that the higher the serum-cell ratio, the higher the tendency for red cell lysis.[14] Worlledge *et al*.[9] used 1% suspension of red cells in saline while 5% red cell suspension in saline was used in this study which may account for the higher prevalence found by Worlledge *et al*. compared with this study. Geographical location has also been proffered as a possible reasons for variations in prevalence obtained from various parts of Nigeria.[10,12]

A higher prevalence may have been obtained if the screening had included the use of a spectrophotometer although one may infer that those positive only by spectrophotometer will not be of danger of clinical hemolysis.

Anti-A hemolysins were found to have a higher prevalence than anti-B hemolysins in this study with anti-A and anti-B hemolysins found in 25.1% and 14.9% of 350 donor samples respectively. These results were not consistent with the findings of Olawumi and Olatunji,[8] Worlledge *et al*.[9] and Okafor and Enebe[11] who all found anti-A hemolysins occurring less frequently than anti-B hemolysins.

This study showed that anti-B hemolysins had visual and spectrophotometric titers which were higher than those found in anti-A hemolysins. The arithmetic mean for visual titers for anti-B hemolysins was 7.3 ± 4.1 compared with 6.3 ± 4.7 for anti-A hemolysins. The arithmetic mean for the spectrophotometric titers for anti-B hemolysins was 101.2 ± 64.5 compared with 80.9 ± 64.8 for anti-A hemolysins. The higher titers found in anti-B hemolysins in this study is consistent with the results found by Grundbacher[15] but is not consistent with results found by Adewuyi *et al.*,[6] Olawumi and Olatunji[8] and Worlledge *et al*.[9] who found higher titers for anti-A than anti-B hemolysins.

Anti-A and anti-B hemolysins with visual titer of 8 or higher were found in 10% and 8.6% of donor samples respectively. This is explained by anti-A having a higher prevalence than anti-B in the analyzed samples. 18.6% of samples thus had visual titres of 8 and above which is able to cause significant *in vivo* hemolysis.[13,16] This finding is similar to that reported by Adewuyi *et al*. among black Zimbabweans[6] but higher than that reported by Olawumi and Olatunji who found a significant prevalence titre of 2.0% for anti-A and 2.8% for anti-B[8] and Kagu *et al*. that found prevalence of 0.4% for anti-A and 0.2% for anti-B.[17] Such difference may be explained by the large sample size used by Kagu *et al*. compared with that used in this study. Another explanation for the difference could be the addition of adsorbed fresh O serum to the test serum by Olawumi and Olatunji and Kagu *et al*. While this was meant to serve as a source of complements, the adsorbed fresh O serum will dilute the serum and may lead to a reduction in the titers obtained.

The influence of sex, rhesus D blood group and age on the frequency of hemolysin among the 350 donor sera analyzed showed no significant relationship. The results obtained with sex and ages were in agreement with those found by Adewuyi *et al*.[6] and Olawumi and Olatunji.[8]

Conclusion

This study showed that anti-A and anti-B hemolysins exist in significant frequency in blood donors in Lagos with a number of these donors having significant titers of hemolysins. With the continued frequent transfusion of group O blood to blood groups A, B and AB recipients especially when there is shortage of group specific donor blood and with blood component therapy still not routinely practiced in our environment, there is a need to routinely screen for hemolysins so as to prevent a potential risk to

recipients. Further studies to associate donor characteristics with hemolysin presence and titers and to determine the episodes of hemolysis in non-group O recipients of blood group O are needed so as to correlate the high prevalence with clinical findings.

It is however recommended that the use of group O blood for recipients who are non-blood group O should be discouraged. Measures should also be put in place to ensure blood component therapy becomes routine practice in our environment.

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