Original Article

http://jbrcp.org

2018 Journal Impact Factor: 1.10
Print ISSN: 2636-7378 | Online ISSN: 2651-5865

Prevalence of Anti-A and Anti–B Haemolysins Among Blood Group 'O' Donors in Makurdi, Nigeria.

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Article information
Date Submitted: 31/08/2023
Date Accepted: 18/12/2023
Date Published: 10/01 /2024

ABSTRACT

Blood group O donors are inappropriately called “universal donors.” These donors could become potentially “dangerous” if haemolysins are detected in their plasma. This study determined the prevalence of anti–A and anti–B haemolysins among blood group O donors in Makurdi, Benue State. Three hundred and five voluntary group O donors were screened for anti–A and anti–B haemolysins using the standard tube technique and samples showing haemolysis were titrated for anti A and anti B haemolysins. The overall prevalence of anti–A and/or anti–B haemolysins was 66.2%. Prevalence of anti–A haemolysins was 6%, anti–B haemolysins 14%, and both was 45.6% of blood donors. It was concluded the prevalence of anti-A and Anti-B haemolysins is high among blood group O donors in Makurdi. In high titres, these lytic (lgG) antibodies may induce haemolysis leading to a haemolytic transfusion reaction during blood transfusion.To prevent this potential adverse event, it is recommended that the transfusion of blood group identical units remains the best practice in the blood bank. However, where this is impracticable, blood group O donor units should be screened so that units with high titre haemolysins will be identified and avoided for non-O blood group recipients as well as utilization of washed blood group O red cells in dire situations where group O blood will be used for non-O recipient.

Keywords: Blood Donors, Haemolysins, Makurdi, Prevalence

INTRODUCTION

Group “O” donor blood is readily available and used in many blood banks in Nigeria and other developing countries[1] in addition to being the commonest and the most prescribed blood group type in our environment for both blood group O and non-O blood group recipients (A, B and AB recipients).[1] In 1923, the term “dangerous universal blood donor” was first coined to describe the agglutination potential of erythrocytes of non–O recipients, due to the plasma of blood group O donors that contained high titres of anti–A and /or anti– B lytic immunoglobulin G (IgG) antibodies also known as haemolysins.[2] In high titres, the lytic immunoglobulin G (IgG) antibodies may induce haemolysis during blood transfusion[3] and the occurrence of these anti–A and anti–B haemolysins in blood group O donors has been reported to be high in the

How to cite this article

Aba IH*, Okolie I, Okoli RO, Aba JP, Mke A, Alao OO, Nwannadi AI, Egesie JO, Bitto TT, Blessing KM, Kyoive E. Prevalence of Anti-A and Anti – B Haemolysins Among Blood Group 'O' Donors in Makurdi, Nigeria. J Biomed Res Clin Pract: 2024;7(1):00-00.
DOI: https://doi.org/10.5281/zenodo.10720672

Access to the article
Website: http://www.jbrcp.org
DOI: 10.5281/zenodo.10720672

African population.[4]

Blood fractionation is routine in most developed countries as opposed to what is obtained in sub-Saharan Africa where the commonest blood product used in this region, is fresh whole blood.[5,6,7] However, the practice of fractionation of blood to its various components is still not routine in most Nigerian blood transfusion service centers and hospital blood banks as corroborated by Okoye *et al*, who observed that only 43·4% of blood transfusion centres in Nigeria had a cold centrifuge for component preparation.[6] It means, therefore, that majority of the clinicians in Nigeria, resort to transfusion of group compatible whole blood to recipients who may have otherwise needed only blood components such as packed red cells or fresh plasma.[8,9] Attempts have been made to study the prevalence of these haemolysins in group O donors in various populations with varying results in Nigeria, Africa and the world.[3,4,10-13] Paucity of comparable data on the prevalence of these haemolysins in Benue State, necessitated this study.

MATERIALS AND METHODS

This was a cross-sectional study that was carried out between May and October 2019 at the Benue State University Teaching Hospital (BSUTH) blood bank, in Makurdi, Benue State and three hundred and five consenting blood group O donors were recruited for this study. Inclusion criteria were fitness of the donor and their signed consent for the study. Fitness for blood donation was determined from results of the blood donor screening questionnaire (which assessed donor's biodata and demographics, blood donation history, medical, surgical, vaccination, social and sexual histories as well as obstetric and gynaecological history for females), physical examination (including weight, height and blood pressure) and negative screening results for transfusion transmissible infections (TTIs), mainly Human Immunodeficiency Virus (HIV), Hepatitis B and C and Syphilis.

Ethical approval was obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital.

Five millilitres of haemoglobin-free serum were obtained from clotted and centrifuged samples and these were stored at minus -20^{o}C until analyzed. The blood samples were then screened for anti–A and anti–B haemolysins using the standard tube technique at 37^{0}C for one hour.

Haemolysin Determination

The haemolytic properties of IgG anti–A and anti–B was adopted for this test.[14]

A volume of donor serum was placed into each of three test tubes and a five percent suspension in normal saline of known red cells of groups A, B and O 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 one hour. The supernatant for each tube was then examined macroscopically and microscopically for haemolysis. [1,11]

Haemolysis found in the tubes was graded as follows: 3+ = complete haemolysis, 2+ = partial haemolysisi.e greater than 50% but not complete, 1+ = trace haemolysis, and negative = no visible haemolysis.

In the samples with haemolysis, anti-A and anti-B haemolysins were titrated for thus:

150µl of donor sera positive for haemolysis were placed on the first row of a sterile 96 well round bottomed microtitre plate (i.e neat serum) and each serum was serially double diluted in normal saline to a titre of 1024. 150µl of 5% washed red cells suspension (A or B and O) were each added to the wells. The O cells suspension served as a negative control and the well containing it served as serum blank well. All microplates were incubated at 37°C for one hour. The supernatant of each well was then examined for haemolysis.The last serum dilution where haemolysis was noted was taken as the haemolysin titre.

Following visual assessment of haemolysis, another set of microplates were set up corresponding with the ones above. 120µl of saline was placed into each well. 30µl of the supernatant from the corresponding incubated wells above were 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 540nm using normal saline as blank. The spectrophotometric titre was taken as the last dilution which does not have the same optical density as the serum blank.[1,10]

Results obtained from the study were analyzed using the IBM SPSS (Statistical Package for the Social Sciences) version 20.0. Results were presented as mean values (±SD). Means of quantitative variables were compared using paired samples. T–test and chi–square tests were used to test for associations between qualitative variables. The level of statistical significance was set at $p<0.05$.

RESULTS

Three hundred and five blood group O donors were screened for anti–A and anti–B haemolysins. The donor ages were between 18 and 55 years with a mean age of 28.0±7.7 years. There were 226 males and 79 females (Table 1).

Overall,66.2% of donor samples had anti–A and/or anti–B haemolysins. Six percent (6%) had anti-A haemolysins, 14.0% had anti–B haemolysins while 45.6% had both anti–A and anti–B haemolysins as seen in table 2. The prevalence of anti–B haemolysins was also statistically significantly higher than that of anti–A haemolysins in the study population as seen in tables 3 and 4.

Table 1: Sociodemographic characteristics of blood group O donors at the blood bank of BSUTH

Variables	Frequency	Percent
Age Group (Years)		
<21	42	13.8
21 - 25	170	55.7
26 - 30	52	17.0
31 - 35	21	6.9
36 - 40	10	3.3
41 - 45	3	1.0
46 - 50	5	1.6
51 - 55	2	0.7
Mean age of 28.0 ±7.7		
Sex		
Male	226	74.1
Female	79	25.9
Educational Level		
No formal education	5	1.6
Primary	4	1.3
Secondary	80	26.3
Post - Secondary	216	70.8
Marital Status		
Single	219	71.8
Married	82	26.9
Divorced	3	1.0
Widowed	1	0.3
Ethnicity		
Tiv	190	62.3
Idoma	53	17.3
Igede	15	4.9
Igbo	11	3.6
Hausa	2	0.7
Yoruba	2	0.7
Others	32	10.5
Religion		
Christian	302	99.0
Muslim	3	1.0

Table 2: Frequency of anti-A and Anti-B Haemolysins Among Blood Group O Donors

Variable	Frequency	Percent
Both Anti-A and Anti-B	139	45.6
Neither Anti-A nor Anti- B	103	33.8
Anti- B alone	43	14.0
Anti-A alone	20	6.6

Table 3: Visual and Spectrophotometric Titre of Haemolysins among Participants

Statistical Parameters	Visual		Spectrophotometric	
	Anti -A titre	Anti -B titre	Anti -A titre	Anti -B Titre
Mean	7.64	7.66	15.28	15.33
Std Deviation	6.02	4.30	12.15	8.60
Median	8.00	8.00	16.00	16.00
Minimum	2.00	2.00	4.00	4.00
Maximum	64.00	32.00	128.00	64.00

Table 4: Comparison between Mean Visual Titres and Mean Spectrophotometric Titres of Anti-A and Anti-B Haemolysins Among Participants.

				Paired Samples T-Test				
	Mean	SD	Std Error	95% CI of the Difference		T	Df	p-value
				Lower	Upper			
Spec titre anti -A/visual titre anti-A	7.641	6.073	0.470	6.713	8.569	16.259	166	<0.001 **
Spec titre anti -B/visual titre anti-B	7.663	4.297	0.322	7.027	8.298	23.794	177	<0.001 **

****P value is significant at <0.001**

DISCUSSION

Best practice in transfusion medicine recommends transfusion of group identical blood units to recipients after a major crossmatch. That means, group O blood should only be transfused to group O recipients, blood group B to blood group B recipients and so on.[1,12] However, shortage of regular voluntary blood donors in our blood banks and such events, results in shortage or non-availability of all specific blood groups in sufficient quantities when needed, especially in emergency situations.[1]Group O donor blood is often more readily available in our blood banks because group O individuals make up over 52% of the population.[15]Moreso, because the blood group O express neither the A nor B antigens which are targets for the anti-A and anti-B antibodies present in the serum of blood group B and A recipients respectively, they are erroneously called universal donors and therefore, most clinicians utilise blood group O donor blood for transfusion.[1]However, some blood group O individuals develop these lytic IgG antibodies in their plasma hence this practice of utilizing blood group O whole blood as universal blood donor units puts recipients at risk of haemolytic transfusion reactions during blood transfusion from these"dangerous" blood group O donors.[2] Furthermore, hospital blood banks and transfusion services in Nigeria do not routinely fractionate blood into its various constituents. These challenges are due to lack of equipment and poor inclination to donate blood, hence recipients who ordinarily need packed red cells and/or fresh plasma are often transfused group compatible whole blood by their attending doctors.[4]

Routine screening for haemolysins is also not performed in our blood bank but with a background information of high prevalence of haemolysins among blacks and from results obtained from other studies within Nigeria, Africa and the world[3,4,10-13] 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 non group O recipients.

The prevalence of anti-A and/or anti-B haemolysins obtained in this study was 66.2%. This high prevalence of anti–A and anti–B haemolysins among blood group O donors corroborates with the findings of Kagu *et al*[4] who in a study conducted in Maiduguri, Nigeria found a haemolysins prevalence of 55.4%.

However, the study by Oyedeji et al in Lagos Nigeria [1] reported a prevalence of 30.3% while Olawumi and Colleagues in Ilorin[10] found an overall prevalence of haemolysins to be 23.2%. These seroprevalence values are lower than reported findings in this study. The observed differences might be due to differences in serum-cell ratios used in the different studies. For example, it has been widely reported that the higher the serum – cell ratio, the higher the tendency for red cell lysis.[13] In addition to the foregoing, geographical location could also be a possible reason for variation and differences in prevalence obtained from various parts of Nigeria. There could be additional reasons responsible for the observed differences and more studies are needed to elucidate these findings.

This study showed a significantly higher prevalence of

anti–B haemolysins than anti–Ahaemolysins, as participants who had anti-B haemolysin were 1.15 times (59.6%) the number of those who had anti–A haemolysin (51.6%). The results are consistent with findings from Olawumi et al in Ilorin Nigeria[10] which showed that anti–B haemolysin prevalence was double that of anti–A haemolysin prevalence. However, contrary to the findings in this study, some studies conducted in Nigeria have reported a higher prevalence of anti–A than anti–B. Oyedeji and colleagues[1] in Lagos observed that participants with anti–A haemolysin were 1.68 times more than those with anti–B haemolysin.

Similar studies from other countries show some notable similarities and differences from the index study's findings. In a study carried out in Abidjan on 191 subjects[11] for instance, anti–B haemolysin showed higher prevalence (15.71%) when compared to anti–A haemolysin (at 10.47%) values, giving further credence to the findings of this study. However, some other studies are at variance with this observation. A study carried out in Thailand[12] shows slightly higher anti–A prevalence at 1.09 times higher values than for anti–B haemolysin; researchers in India[16] also showed a greater anti–A prevalence while a study conducted in California[17]shows as high as 3.36 times greater prevalence values for anti–A haemolysin (44.8%) than for anti–B haemolysin (13.3%).

These findings suggest that haemolytic transfusion reactions due to high titre haemolysins may be occurring at a higher frequency than is reported hence the need for a preference of transfusion of group identical whole blood units at the BSUTH blood banks, where possible and proper crossmatching procedures to ensure compatibility when group compatible transfusions must be utilised for diverse reasons.

CONCLUSION

The prevalence of anti–A and/or anti–B haemolysins is relatively high among blood group O donors in Makurdi. However, in the face of shortage of group specific donor blood, continued utilisation of group O blood to non-blood group O recipients and an absence of blood component therapy at the BSUTH, the following are the recommendations from this study;

Measures should be put in place to attract and retain large numbers of voluntary blood donors so as to prevent shortage of specific blood groups even in emergencies.

New blood bank policies should be drafted that will encourage blood group identical transfusions at all times.

Blood preparation procedures should be updated to include routine screening of haemolysins in all blood group O donated units.

Efforts should be made to introduce blood component therapy as the most appropriate method of providing safe blood for recipients of blood transfusion or washed red cells as an alternative.

All incidences of transfusion reactions reported to the blood bank should be thoroughly investigated to ascertain a cause and confirm or rule out haemolytic transfusion reactions.

Data collated from these investigations may be used for advocacy for requesting improved blood banking techniques at the BSUTH blood bank especially blood component therapy.

Conflict of Interest

None.

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