

**Research Article****Incidence of Haemolysin among Group ‘O’ Donors Recruited At a Secondary Health Care Facility****Oluwatayo BO<sup>1</sup>, Olayanju AO\*<sup>2</sup>, Edeyokun OA<sup>1</sup>, Enitan SS<sup>3</sup>**<sup>1</sup>Haematology and BGS Department, Federal College of Veterinary and Medical Laboratory Science, NVRI, Vom<sup>2</sup>Department of Medical Laboratory Science, College of Medicine and Health Science, Afe Babalola University, Ado-Ekiti<sup>3</sup>Department of Medical Laboratory Science, Babcock University, Ogun State, Nigeria.**\*Corresponding author**

Olayanju A.O.

Email: [dddickson@yahoo.com](mailto:dddickson@yahoo.com)

---

**Abstract:** Haemolysins are antibodies or Immunoglobulin which in the presence of complement, cause the lysis of the corresponding red cell antigen. High titre alpha ( $\alpha$ ) and Beta ( $\beta$ ) haemolysin in blood of donors shorten the life span of recipient's cells. This institution based, cross sectional study was designed to determine the occurrence of Haemolysins in group O type donors. A total number of 209 sera samples of apparently healthy group O donors were screened for haemolysins, using standard protocols. A cut-off titre value of 16 and 32 was set for  $\alpha$  and  $\beta$  haemolysin respectively. Four (1.9%) of the samples contained  $\alpha$  and  $\beta$  haemolysins, and in one case (0.5%), only  $\alpha$ -haemolysin was detectable in high titer. In this study, the incidence of haemolysin among group O donors was low; therefore routine screening may not be necessary. However, when an elective transfusion of O blood group to a non O group individual is anticipated, it is important to exclude the presence of haemolysin before selecting compatible units**Keywords:** non group transfusion, haemolysis, haemolysin, group O donors.

---

**INTRODUCTION**

Blood transfusion is an essential form of medical treatment, since common illnesses are usually related to blood destruction or blood loss[1]. There is a huge requirement for the use of blood and its products in clinical care. The practice in most parts of the developing world is fraught with the problem of an inefficient blood banking system[2]. In addition, facilities for the extensive screening of blood prior to use are highly limited; hence, the risk of transmission of infections like hepatitis, cytomegalovirus, syphilis, and HIV is high[3]. In addition to this, the standard protocols to ensure the compatibility of the recipients' blood with donors are often abbreviated or omitted.

Allogenic blood is of high demand in most developing countries including Nigeria and the shortages have resulted in the increasing transfusion of group O blood Type as 'universal donors' to non group O individuals [4]. Previous report however advocate that the use of O blood for A or B recipients be discontinued considering the high frequency rate of alpha and beta haemolysins among their cohort of group O donors[5]. Despite these concerns the transfusion of blood group O to A or B recipients has continued on the assumption that there are no clinically significant adverse effects.

Blood group- O individuals can have potent high titre anti-A and anti-B antibodies (haemolysins) in the serum. These high titre anti-A (alpha) and anti-B (beta) haemolysins are capable of causing haemolytic transfusion reaction when given to A, B and AB patients following complement activation. As a rule, all group O blood red cells that are intended for use against ABO blood group barrier in blood group A, B and AB individuals must be tested for high titre anti-A and B haemolysins. Only those that are negative should be used for group A, B and AB recipients. Those that are positive for anti A and B haemolysins should be reserved strictly for recipients who are group O [6]. This rule is not observed in most hospital facilities, hence the need to determine if the incidence of haemolysin is high enough to include the test as part of the investigations during compatibility testing in our locality.

**MATERIALS AND METHODS****MATERIALS**

Two hundred and nine (209) voluntary group "O" donors, at Plateau State Specialist Hospital Jos who had been screened, found fit and accepted as donors were included for the screening. Five (5) mls of blood was collected from the antecubital area, by vene-puncture into suitable dried clean containers. Haemoglobin-free serum was obtained, by centrifugation of whole blood at 2500 rpm for five minute. 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.

**METHODS**

**ABO BLOOD GROUP TYPING**

Rapid ABO grouping typing was done using polyclonal anti A, anti B and anti AB sera. On clean grease free slide, equal volume of the anti sera and 5% suspension of the test and control cells was added and mixed together. The tile was rocked and observed within five minutes for the presence of agglutination [7].

**TEST FOR HAEMOLYSIN**

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 1 volume of 5% suspension in saline solution of red cells of group A, B, and O, respectively[8]. The O red 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. The content of each tube was mixed thoroughly, and incubated at 37°C. At 1 hour, the samples were examined for haemolysis visually and microscopically. Titres were recorded as the reciprocal of highest dilution showing the weakest haemolysis microscopically.

**DETERMINATION OF HAEMOLYSIN/ ANTIBODY TITRE**

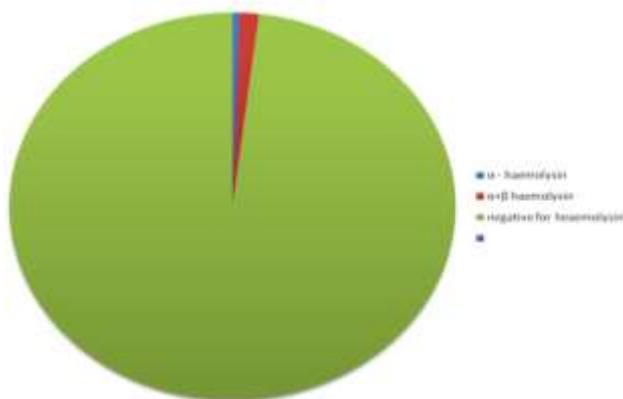
15 dry precipitin tubes were arranged in a metal rack. To tube number one and two were added, one volume of the serum under investigation. To tube numbers two (2) to fifteen (15) were added one volume of normal saline. The doubling dilution was done by mixing the content of tube number 2, and then transfers 1 volume of the mixture to tube number 3. This process was repeated up to tube number 15 in which one volume were discarded from tube number 15. To each of the tubes were added five percent standard ABO cells and incubated for 2 hours at room temperature.

**ETHICAL CLEARANCE**

The Research Ethics Committee of the Plateau State Specialist Hospital Jos approved the research protocols. Informed consent was obtained from the subjects. Only donors who gave consent were recruited for the study.

**RESULTS**

Only 4(1.9%) out of 209 subjects screened were found to posses Heamolysin. One of this 4 had only alpha (α) haemolysin, while the remaining 3 (1.4%) had both alpha (α) and Beta (β) haemolysins (Figure 1).



**Fig 1: sero-prevalence types of haemolysin detectable at significant titre in the blood donors**

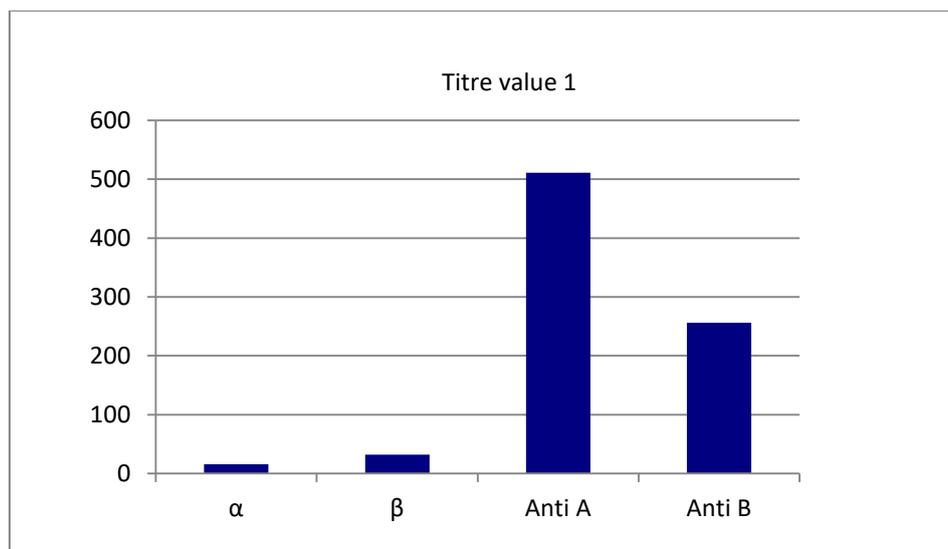


Fig 2: Mean Antibody Titre of  $\alpha$ ,  $\beta$ , Anti A and Anti B of Blood donors

## DISCUSSION

A policy of transfusing only group identical donor blood to recipients would make the screening of blood for haemolysin unnecessary. However, the non availability of donor blood of all groups at all times necessitates the transfusion of group O donor blood to certain recipient of A, B and AB blood group particularly in developing countries.

The present study shows incidence of alpha and beta haemolysin among group O donors in Plateau State Specialist Hospital, Jos. The incidence (Figure 1) was 1.9% among 209 blood group O donors screened,  $\alpha$ , 1(0.5%),  $\alpha+\beta$  3(1.4%). This is slightly lower compared to those reported by Isahaku (2.4%)  $\alpha$  (0.4%),  $\beta$  (0.8%)[9], and  $\alpha+\beta$  (1.2%) in 2005, Olawunmi and Olatunji (23.2%) in 2001[3] and Emeribe (30.6%) in 1990[5].

In this study, the titre value for  $\alpha$  haemolysin and  $\beta$  haemolysin was 16 and 32 respectively. Those that do not possess haemolysin, have titre values different from those with haemolysins (Figure 2). According to Reed SG[10], titre value of anti A is between 128 and 512, while titre value of anti B is 256. The subjects had a mean titre value of 512 for anti A and 128 for anti-B. Incompatible ABO blood group transfusion is major cause of transfusion reactions. It is important that the National Blood Transfusion Service Centres in the Zones/States maintain adequate stock of all blood types and products to meet the increasing demand for safe allogenic blood transfusion. Haemolysis as a consequence of haemolysin in transfused units will not occur if a policy of transfusing only identical blood types to recipients is observed. Where this is not possible, compatible blood group O units should be pre-screened for haemolysin. Group O blood should be transfused as plasma reduced blood or packed cell in cases where the blood transfusion is indicated for the management of anaemia only.

This study shows that percentage of haemolysin of group O donors 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. This is important to prevent transfusion reaction.

## REFERENCES

1. Gorst DW; Haemorrhagic disorders and the use of blood products. In: Hendrickse RG, Barr DG, Matthews TS, editors. Paediatrics in the Tropics. 1<sup>st</sup> ed, Oxford: Blackwell Scientific Publications; 1991; 360-72.
2. Enosolease ME, Imarengiaye CO, Awodu OA; Donor blood procurement and utilization at the University of Benin Teaching Hospital, Benin City. Afr J Reprod Health 2004; 8: 59-63.
3. Ogunlesi TA, Ogunfowora OB; Pattern and determinants of blood transfusion in a Nigerian neonatal unit. Niger J Clin Pract 2011; 14: 354-8.
4. Kagu MB, Ahmed SG, Askira BH; "Utilization of blood transfusion service in north eastern Nigeria," Highland Medical Research Journal, 2007; 5(2): 27-30.
5. Emeribe AO; The status of alpha and beta haemolysins in Nigerian blood donors. East Afr Med J. 1990; 67(3): 205-208.
6. Uko EK , Erhabor O , Ahmed HM , Isaac IZ , Abdulrahman Y , Wase A, Ezimah A; Prevalence of high titre alpha and beta haemolysins among blood donors in Sokoto, North Western Nigeria. International Journal of Medical Sciences and Health Care. 2013; 1(11): 1-8.
7. Dacie JV, Lewis SM; Practical Haematology, 7th ed. Churchill Livingstone Edinburgh; 2001; 281-283.
8. Crawford H, Cutbush M, Falcorner H, Mollison PL; Formation of immune A iso-antibodies with special reference to heterogenitic stimuli, Lancet 1952; 2:219-223.
9. Salleh WM, Ahmad F, Yen KH; Chemical compositions and biological activities of the essential

oils of *Beilschmiedia madang* Blume (Lauraceae).

Archives of pharmacal research, 2014; 38(4):485-493.

10. Reed SG, Lodes MJ, Mcneill PD, Houghton RL; U.S. Patent No. 20,020,086,984. Washington, DC: U.S. Patent and Trademark Office. 2002.