

Epidemiology of Haemolysins among Blood Donors in Abakaliki, Ebonyi State, Nigeria

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Abstract

Anti-A and anti-B haemolysins are lytic antibodies that cause haemolysis of red blood cells of recipients when 'O' group blood or plasma is transfused to non 'O' group patients. They are known to cross the placenta and cause haemolytic disease of the newborn. These antibodies are large fractions of A and B antibodies that belong to IgG and IgM class of antibodies. Most blood group O individuals that are over the age of 6 months possessed anti-A and/or anti-B in their serum if they lack the corresponding A or B antigens on their red cells. These antibodies may become clinically significant high titre immune antibodies. The occurrences of anti-A (alpha haemolysin) and anti-B (beta haemolysin) in group O donors have been reported to be high in African population. The prevalence of haemolysins among group O donors in Federal Teaching Hospital, Abakaliki was determined using haemolysin assay and antibody titration. Data obtained were analyzed statistically using descriptive statistics and inferential statistics of Chi-Square. One hundred and fifty seven samples from group O donors comprising 126 (80.25%) males and 31 (19.75%) females were enrolled for this study. Among the overall population studied, a total of 83 (52.87%) had alpha and/or beta haemolysin. Alpha haemolysin was found in 33 (39.76%), 26 (31.33%) had beta haemolysin while 24 (28.91%) had both alpha and beta haemolysin. Among the 83 samples positive for haemolysins,

52 (62.65%) had significant titre (visual titre of 8 and above) while a total of 53 (63.85%) had visual titre. This study advocates the need to routinely screen for haemolysin whenever a group O donor blood is to be transfused to a non-group O recipient.

Keywords: Haemolysin; Donors; Blood group O; Titre; Haemolysin assay

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Introduction

Anti-A and anti-B haemolysins are lytic antibodies that cause haemolysis of red blood cells of recipients when 'O' group blood or plasma is transfused to non 'O' group patients. They are known to cross the placenta and cause haemolytic disease of the newborn [1]. These antibodies are large fractions of A and B antibodies that belong to IgG and IgM class of antibodies [2]. Variable proportions of dangerous group O donors have been reported and in most studies it is found that approximately 10-20 percent of group O donors have high titre of anti-A or anti-B haemolysins [3-5].

The practice in blood banks with inadequate blood supply is to use group O donor blood as a universal donor where group identical blood is not available or in the transfusion of infants born to non-group identical mothers [6]. It has long been recognized that certain blood group O donors possess potent ABO antibodies (anti-A and anti-B haemolysins) in their plasma, which are dangerous to the recipient's red blood cells [7]. These haemolysins are found in the blood after allogenic stimulation by red blood cell-like antigens (A-like and B-like antigens) derived from tetanus toxoid, antitetanus serum and typhoid-paratyphoid A and B vaccine [8]. That is why the universal blood donor phenomenon has been outdated in major blood banks.

The importance of 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 red cells in vivo [9]. 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 blood cells [9]. Blood group O red blood cells can be given to A, B or AB recipients and were formerly inappropriately called "universal donor red blood cells" [10]. However, early studies have shown high frequency of potentially lytic anti-A and anti-B haemolysins in blood group O persons [10, 11]. This 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 [12, 13].

The occurrence of anti-A (alpha haemolysin) and anti-B (beta haemolysin) in group O donors was reported to be high in African population [14-16]. As such, some laboratories still spend time and resources screening for these lytic haemolysins using the labour-intensive technique due to the non-availability of any automated method at present. In 1900, Karl Landsteiner reported a series of tests that identified ABO blood group system, which is known as the most clinically significant blood group system till date [17, 18]. This is so because ABO antibodies are consistently, predictably and naturally present in the serum of people who lack the antigen. ABO compatibility between the donor and recipient is crucial. Hence, strong

naturally occurring anti-A and anti-B haemolysins are IgM and IgG and can readily activate complement and cause agglutination. If ABO antibodies react with antigen in vivo, the result is acute haemolysis and possible death. The clinical significance of ABO blood group antigens relate to the capacity to elicit the production of alloantibodies (both IgM and IgG) to cause destruction of transfused red cells or to cross the placenta and give rise to haemolytic disease of the newborn [19].

There are reports of acute intravascular haemolysis after transfusion of incompatible group O blood/ plasma components [5, 20-24]. In Nigeria, as adequate supply of blood is not available, it is not uncommon to transfuse group O blood to non group O patients. With limited transfusion work up, passively transferred antibodies in group O blood/plasma could result in intravascular haemolysis in such patients.

The prevalence of alpha and beta haemolysins has been reported in the range of 30-56% [6, 11, 25]. 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 [6]. The haemolysin screening test to identify dangerous group O blood containing the potent haemolysin in the donor pool is performed routinely in some blood banks. However, some other blood banks including Federal Teaching Hospital, Abakaliki do not carry out the test despite the reported higher prevalence of haemolysins among group O donors. This study aimed at determining the prevalence and titre of alpha and beta haemolysins among blood donors in Abakaliki metropolis.

Materials and Methods

Study Area and Sample Population

The study was undertaken in Abakaliki, the capital of Ebonyi State, South Eastern Nigeria. One hundred and fifty seven samples from group O blood donors comprising 126 (80.25%) males and 31 (19.75%) females were enrolled for this study after signing the informed consent form presented to them. The ages of the donors ranged from 19 to 49 years. The participants were selected using simple random sampling technique. The samples were examined for anti-A and anti-B haemolysins. A total of 9ml of blood samples were collected from the subjects,

4ml was dispensed into EDTA bottle while 5ml was dispensed into plain bottles for serum extraction.

Five (5ml) of whole blood samples were obtained from group O donors who had been screened, found to be eligible to donate blood and had been accepted as donors in the blood bank of Federal Teaching Hospital, Abakaliki. These samples were preserved in EDTA containers. At least 2ml of haemoglobin free serum was obtained from clotted samples after centrifugation and stored in the refrigerator for haemolysin assay. Whole blood samples of blood group A and B were also obtained and preserved in EDTA containers and refrigerated at 4°C for the assay. Before use, the red blood cells were washed four times in normal saline and the washed red blood cells obtained and used for the haemolysin assay.

Blood Group Assay

Blood group assay was carried out. Whole red blood samples were used to carry out ABO blood grouping by tube method using commercially prepared antisera supplied by BioTech Laboratories Ltd, UK.

Haemolysin Assay

Fresh A and B cells were washed four times in saline and red cell suspension was made. Three tubes were arranged and two parts of washed A, B and O cells were dispensed into each of the two tubes, the O cells were used as negative control. Four parts of group O serum was added to the tubes and then they were incubated at 37°C for 30 minutes. Subsequently, the tubes were centrifuged at 2500rpm for 1 minute and then they were held before a source of light and with minimal disturbance, the supernatant was observed for haemolysis macroscopically, followed by microscopic examination using 10x and 40x objective lenses respectively and the results were recorded. Haemolysis was graded as follows; complete haemolysis 3+, partial haemolysis 2+, trace haemolysis 1+, and no visual haemolysis, negative.

Antibody Titration

All the samples showing haemolysis were titrated for anti - A and anti - B haemolysins. 2ml of each serum was double diluted serially in saline up to 64, and 0.5ml of each serum dilution and

0.5ml of absorbed fresh group O serum were placed in each of the 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 negative control. All tubes were incubated at 37°C for 1 hour. At the end of incubation they were centrifuged and the supernatant examined visually for haemolysis. The visual titre was taken as the last dilution of serum where haemolysis was seen.

Data Analysis

The data generated were analyzed using simple descriptive statistics and inferential statistics of Chi-Square. Statistical analysis was performed with the aid of Statistical Programme for Social Sciences (SPSS) version 18.0.

Results

Among the overall population studied, a total of 83 (52.87%) had alpha and/or beta haemolysin. Alpha haemolysin was found in 33 (39.76%), 26 (31.33%) had beta haemolysin while 24 (28.91%) had both alpha and beta haemolysin (Table 1). 54 (65.06%) samples had haemolysis of grades 3+ and 2+ against both A and B cells whereas 29 (34.94%) had trace haemolysis.

Table 1: Prevalence of Haemolysins among the Population Studied

Haemolysin	Number (n)	percentage (%)
Alpha	33	39.76
Beta	26	31.33
Alpha+ beta	24	28.91
Total	83	100

Antibody titres were observed to be higher for anti-B than for anti-A. The minimum titre for both anti-A and anti-B was 1 while the maximum titre was 8 and 16 for anti-A and anti-B respectively. Among the 83 samples positive for haemolysins, 52 (62.65%) had significant titre (visual titre of 8 and above) while a total of 53 (63.85%) had visual titre (Table 2). The titres were higher among samples with both alpha and beta haemolysins when compared with the positive titres of samples with either alpha or beta haemolysins. There was no significant

influence of sex or age on the frequency of haemolysins observed in this study.

Table 2: Visual Titres of Anti -A and Anti-B Haemolysins among the Donor Samples with Haemolysins

Dilution	Anti-A	Anti-B
1	5	7
2	8	6
4	11	8
8	3	4
16	0	1
32	0	0
64	0	0
Total	27	26

Discussion

High frequency of strongly haemolytic anti-A and anti-B haemolysins has been reported from Asian and African populations as compared to Caucasians [10, 11, 26, 27]. The higher grades of haemolysins in these populations have been attributed to mosquito bites and intestinal parasitic infections [28]. High titres of ABO haemagglutinins in O group individuals can also be the consequence of vaccination or other antigen exposures [29].

The result obtained from this study revealed a haemolysin prevalence of 52.87% in the 157 blood group O donors studied. This relatively high overall haemolysin prevalence is comparable to the reports of other workers from Nigeria. An overall prevalence of 55.4% was found by Kagu and colleagues [30], while 30.6% and 53.6% were found by Okafor and Enebe [14] and Emeribe [15] respectively. However, a lower prevalence of 23.2% was found in the study by Olawumi and Olatunji [16]. It was observed in this study that beta haemolysin occurred less frequently compared with alpha haemolysin. This is consistent with the finding of Adewuyi and colleagues [27] among black Zimbabweans.

Visual titre was observed to be higher among samples with both alpha and beta haemolysins when compared to the positive titres of samples with either alpha or beta haemolysins. Taking a

visual titre of 8 and above as being able to cause haemolysis in vivo [6], it was observed that antibody titres were higher for anti-B than anti-A. This is in contrast to the study by Olawumi and Olatunji [16] which found higher titres for anti-A than for anti-B. This difference may be attributed to the observation that in this study, the donors were predominantly from the Igbo race unlike their study which involved multi racial subjects. This further shows that there is relatively high probability that when transfused, in vivo haemolysis may occur. Of importance in this study is the observation that when alpha haemolysin occurs in combination with beta haemolysin there is higher titre of significant antibodies.

Creating a policy of transfusing only group identical blood may be the best approach for preventing post transfusion haemolysis but this policy will entail improving the amount of all the blood groups in the blood bank at any time. However, where this is not possible, transfusing mainly packed cells from group O donors to recipients of other blood groups may be a safer method.

This study has shown that the prevalence of haemolysin is high in our voluntary group O donor population. Therefore, despite the labour intensiveness of haemolysin titration technique and the frequent transfusion of group O blood to recipients of blood group A, B and AB in our environments, there is 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 group O is needed so as to justify the clinical significance of such antibodies. Also, close monitoring of all transfusions is necessary to prevent significant consequences of such transfusions.

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