PREVALENCE AND PATTERN OF HAEMOLYSIN ANTIBODIES IN PREGNANT WOMEN ATTENDING ABUBAKAR TAFAWA BALEWA UNIVERSITY TEACHING HOSPITAL, BAUCHI

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ABSTRACT

Background: Alpha (α) and Beta(β) haemolysin antibodies have been implicated in blood group O pregnant women as the etiologic agent leading to haemolytic disease of new born. Previous studies in some parts of Nigeria have demonstrated the presence of α and β –haemolys in antibodies among blood group O donors, but there is paucity of data on its prevalence in pregnant women despite the fact that it has been attributed to be responsible for the high prevalence of ABO-haemolytic disease of the newborn seen in Africans.

Aim: To determine the prevalence and pattern of haemolys in antibodies among blood group O pregnant women attending antenatal clinic of Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) Bauchi, for the presence of potentially haemolytic antibodies.

Materials and Methods: Two-hundred and twenty-four (224) pregnant women aged 17 to 40 years were randomly selected and their blood groups were determined, of which one hundred and ten (110) subjects with blood group O were screened for α and β haemolysins using standard tube technique at 37^{o}C, and the results were read macroscopically after 1hour.The degree of haemolysis was graded as complete partial or trace and no visual trace of haemolysis.

Results: Of the two-hundred and twenty-four (224) samples collected, 110(49.1%)were group O, Of the 110 blood group O samples screened, 46(41.8%) showed the presence of haemolysin, of which 20(43.5%), 18(39.1%) and 08(17.4) are alpha(α), Beta(β) and Alpha + Beta(α+β) haemolysins respectively. Complete haemolysis was recorded only in Beta (β) type haemolysin.

Conclusions: This study has shown that the overall prevalence of haemolysins among blood group O pregnant women attending antenatal clinic of ATBUTH is41.8% which is high. The screening of haemolysins in blood group O pregnant women is very important so as to identify women posing great risk to their unborn offspring.

Keywords: Pregnant women, blood group O, Alpha and Beta haemolysin, ATBUTH, Bauchi.

INTRODUCTION

The ABO blood group system is the most important blood type system (or blood group system) in human blood transfusion. The associated anti-A antibodies and anti-B antibodies are usually IgM antibodies, which are usually produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses. The importance of a 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 cells in vivo(Reid, 2013). 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 cells (Reid, 2013).

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Blood group "O" red cells can be given to A, B, or AB recipients and were formerly inappropriately called "Universal donor red cells". Early studies from Nigeria have shown high frequency of potentially lytic anti-A and lytic anti-B in blood group "O" persons(Olawumi & Olatunji, 2001; Oyedeji *et al.*, 2015; State *et al.*, 2013; Zama and Erhabor, 2017). 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. Blood group "O" is the commonest and most prescribed blood group type in our environment(Kagu *et al.,*2011).The occurrence of anti-A (alpha-haemolysin) and anti-B (beta-haemolysin) in group "O" donors was reported to be high in African population (Emeribe, 1990). The prevalence of these haemolysins also varies with age and gender. Kagu *et al.*, (2011)reported a high prevalence of alpha and Beta haemolysin among blood group O donors in North eastern Nigeria. A recent study by Obisesan *et al.,* (2015)also reported a high prevalence of alpha and Beta haemolysins among blood group O donors at ATBUTH Bauchi. However, there's scarcity of data on theprevalence of α and β haemolysins among blood group O pregnant women despite the reported cases of ABO-haemolytic disease of the newborn, though it is generally agreed that hemolytic disease of the newborn is usually caused by antibodies formed in Rhesus-negative mothers against the Rh factor D (Rh0) of Rhesus-positive children, a study in YaoundéCameroon by Sock *et al*(2020)investigated neonatal jaundice due to feto-maternal ABO incompatibilities and to determine the link between the hemolysins value in the mother and the degree of jaundice observed in the infant shows that hemolysins frequency was of 20.58% and anti-A hemolysin was the most common type 85.7%. The new-born who had blood type B had a greater concentration of bilirubin levels compared to those of the AB group. However, a study byUsanga and Akwiwu, (1990)in an effort to determine the prevalence and titre of ABO haemolysin antibodies in pregnant and non-pregnant women in a Nigerian community, the result show that alpha haemolysin occurs less frequently than beta-haemolysin in both pregnant and non-pregnant subjects. However, the prevalence of alpha haemolysin was significantly higher in pregnant subjects than in the non-pregnant ones. Surprisingly, the prevalence of haemolysin was significantly lower among the pregnant group than the non-pregnant group. We aimed to screen blood group "O" pregnant women attending antenatal clinic of ATBUTH Bauchi, to determine the prevalence and pattern of α and or β heamolysins in their blood to find out the

MATERIALS AND METHODS

Study Area

This present research work was carried out at the department of haematology and blood transfusion services of ATBUTH Bauchi Northeaster Nigeria. Subjects were recruited from the pool of pregnant women attending antenatal clinic at ATBUTH Teaching hospital Bauchi Nigeria. It is a 650-bed capacity tertiary hospital that provides multi specialized services and a major referral Centre in the north eastern geographical zone of Nigeria, a region where Neonatal Mortality rate was reported to be relatively high.

Study design

This was a hospital-based cross-sectional study designed to determine the Prevalence and Pattern of alpha and beta haemolysin among pregnant women attending antenatal clinic at ATBUTH Bauchi. Written informed consent were obtained from each subject after counselling. The study protocol was approved by the institutional review board, before commencement.

Study population

The subjects were randomly selected and recruited from the pool of pregnant women attending antenatal clinic of ATBUTH Bauchi

Inclusion and exclusion criteria

Pregnant womenattending antenatal clinic at ATBUTH during the study period identified to have blood group O were included and Pregnant women attending ATBUTH antenatal clinic identified with blood group other than O were excluded.

Sample Size Determination

The sample size was determined using the Leslie Kish formula for cross-sectional studies, where a sample size of 263.5 was obtained using a prevalence of 22.2% from a previous studyObisesan*et al.*, (2015). A total of 264Pregnant women were recruited for this study, however, only 229 participants consented giving 86.7% response rate. Therefore,229 blood samples were collected, of which only 224 were found to be acceptable for testing.

Statistical analysis

Statistical analyses were conducted usingMicrosoft excel 2007 and SPSS (version 11; SPSS Inc., Chicago, IL) software Continuous variables were described using means and standard deviations whereas categorical variables were described using counts and percentages.

Sample Collection

Sterile needle and syringes were used to collect blood samples aseptically,the skin was cleaned in concentric circles of increasing diameter with 70% ethanol and left to dry.(5mls)of blood was drawn from the vein of each subject by venipuncture and dispensed into 2 tubes; EDTA anticoagulated tube (2mls) and plain tubes without anticoagulant (3mls).The EDTA anticoagulated tube was used for the confirmation of ABO blood group of the subjects while the samples in the plain tubes were allowed to clot and centrifuged to obtain hemoglobin-free serum. Each tube was labeled using a unique Identifier (ID), Testing was done within 4 hours of serum separation to prevent the deterioration of serum due to denaturation of complements which can occur due to storage.

Methods

ABO blood grouping

The blood group of the participating subjects was determined using slides method. (Roman, L., Armstrong, B., and Smart, E. (2020).One end of a slide is labelled Anti-A, and the other Anti-B. A drop of Anti-A test serum is added to the end marked Anti-A, and a drop of Anti-B serum is added to the end marked Anti-B One drop of blood is added to each end of the slide, and mixed well, using separate wooden sticks. The results are read directly from the slide. The subject is blood group A if agglutination occurred with the Anti-A test serum; group B if agglutination occurred with the Anti-B test serum; group AB if agglutination occurred with both test serums, and O if there was no agglutination in either case.

Washing the Red Blood Cells

2ml of blood group A or B is added to 8ml of Normal saline solution (0.85% NaCl) in a test tube.The tube was shaken gently to suspend cells. the preparation was centrifuged at 3000 revolution per minute (rpm) for 5 minutes. The supernatant fluid was decanted leaving the cells deposit. Another 8ml of normal saline solution was added and centrifuged Again at 3000 rpm for 5minutes. The process was repeated again until a clear supernatant was Obtained. The clear supernatant was drawn and thrown away. The deposit was used and 5% cells suspensions was prepared. According to Klein and Anstee, (2005)

Haemolysin test (Standard Tube Technique

Kahn tubes were arranged in a rack in duplicate and labeled accordingly. Four volumes of serum from each pregnant woman were placed into each Kahn tube. A volume of pooled, washed, 5% A cells was dispensed into one of the duplicate khan tubes for each sample. A volume of pooled, washed, 5% B cells was dispensed into the other khan tubes for each sample. The preparations were allowed to stand for one (1) hour at 37^0c in water bath incubator

The tubes were tapped to resuspend the cells and centrifuged at low speed for 2 minutes. The solutions were then examined macroscopically in a bright light background for haemolysis, (Dacie JV, Lewis S.M. 1994)

The presence of haemolysis was indicated by the pink (pale- deep) colored supernatant. The same procedure was carried out with AB serum and albumin as positive and negative controls respectively.The degree of Heamolysis was graded as follows

Complete Heamolysis ……... (3+)
Partial Heamolysis (more than 50%) ……... (2+)
Trace Heamolysis ……... …… (1+)
No visual Heamolysis ……. Negative

Known A and B red cells were used to test the subject's sera for agglutination and subsequent hemolysis of the red cells in the presence of haemolysin and complement in the fresh sera samples. (Dacie JV, Lewis S.M 1994).

Samples were observed both physically and via the microscope (visual haemolysis and microscopic agglutination).

RESULTS

Of the two-hundred and twenty-four (224) samples collected,110(49.1%)were group O,48(21.4%) group A, 37(16.5%) group B and 29(12.9%) group AB. The 110 blood group O samples were screen for the presence of alpha(α)and or Beta(β) haemolysin.

Haemolysin Screening Test Result

Table 1. General distribution of haemolysin in blood group O subjects

Subject	Number	Percentage (%)
Haemolysin Positive	46	41.8
Haemolysin Negative	64	58.2
Total	110	100

Table 2. General Pattern of Distribution ofHaemolysin

Haemolysin Type A	A	B	α +β	Total
Number of Positive	20	18	08	46
Percentage (%)	43.5	39.1	17.4	100

Table 3. Degree of Haemolysis (Alpha (α) Haemolysin)

Degree of traced Haemolysis	1+	2+	3+	Total
Numbers of positive	0	8	12	20
Percentage (%)	0	40	60	100

Table 4. Degree of Haemolysis (Beta (β) Haemolysin)

Degree of traced Haemolysis	1+	2+	3+	Total
Numbers of positive	1	7	10	18
Percentage (%)	5.5	38.9	55.6	100

Table 5. Degree of Haemolysis (Alpha (α) and Beta (β) Haemolysins)

Degree of traced Haemolysis	1+	2+	3+	Total
Numbers of positive	0	2	6	8
Percentage (%)	0	25.0	75	100

DISCUSSION

ABO incompatibility between a mother and herbaby is a common and a generally cause of mild type of haemolytic disease of the foetus and newborn.Significant problems with ABO incompatibility occur mostly with babies whose mothers have O blood type and where the baby is either A or B blood type (Zama and Erhabor, 2017).Considerable number of instances of the disease attributed to maternal anti-A or anti-B antibodies has been recorded all over the world(Basu *et al.*, 2011).In this study 110 pregnant women ranging from the age of 17 to 40 years, attending antenatal clinic of Abubakar Tafawa Balewa University Teaching hospital, Bauchi Nigeria, were screened for alpha and beta haemolysins antibodies.This study confirms the presence of haemolysins in Nigerian pregnant women. The overall prevalence was 41.8%, Out of which20(18.0 %) were (α) Anti A haemolysin positive, 18(16.4 %) were (β) Anti B haemolysin positive, and 08(17.4) were α+β positive. This is comparable to the work of Kagu *et al.,*(2011) whose findings show The overall prevalence of haemolysinswas 55.4% while prevalence of alpha- and beta-haemolysins only was 10.3% and 12.6%, respectively" in a voluntarygroup "O"donorpopulation in North eastern Nigeria but contrary to the findingsUsanga and Akwiwu, (1990), whose work shows that "alpha haemolysin occurs less frequently than beta-haemolysin in both pregnant and non-pregnant subjects" this may be attributed to the fact that only pregnant women were screened, also this work contradict the work of Emeribe(1990),who foundthat "the frequency of group O donors with alpha haemolysin only in their sera was 5.7% and beta haemolysin occurring alone in Group O subjects was 8. 6%". butOlawumi and Olatunji (2001) reported that "hemolytic anti A was higher than hemolytic anti B in blood group O donors",which is in conformity of this study's findings. The findings ofUsanga and Akwiwu, (1990) suggest that the fetus probably plays a dual role in alpha and beta-haemolysin production it enhances the production of alpha-haemolysin while suppressing that of beta-haemolysin.Sawadogo *et al,* (2020) in a recent study in Burkina Faso reported that the incidence of β- and α +β haemolysins, are higher than α-haemolysin among blood group O donors, likewise, Oluwomi Olatunji (2001) reported that haemolytic anti B occurred twice as frequent as haemolytic anti A was higher than haemolytic anti-B. which contradicts this study which shows that alpha-haemolysin has a highest incidence. This could be attributed to the geographical location, however another study by Sock *et al* (2020) in Yaoundé among mothers who were blood type O with children who were a different blood type reveled that alpha-haemolysin is most frequent type of haemolysins encountered.

There was no significant age difference in the prevalence of haemolysins this study this is consistent with the finding of Kagu *et al.* (2011),Olawumi and Olatunji. (2001),Adewuyi *et al.* (1994) and Emeribe(1990).

CONCLUSION

This study has confirmed the high frequency of haemolysins in Nigerian group "O pregnant women. The screening of alpha and beta haemolysin in group O pregnant women is necessary so as to determine those women posing greatest risk to their unborn offspring.

RECOMMENDATIONS

A close follow up throughout pregnancy is required to detect irregular antibodies. Therefore, there is need to routinely screen pregnant women for haemolysin antibody in order to identify those posing the greatest risk to their foetus. Further study to determine the episodes of ABO HND and neonatal jaundice in newborns to justify the clinical significance of such antibodies

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Conflict of Interest

No conflicting interest

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