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Seroprevalence of antibodies to human platelet antigens in pregnant women in OSOGBO

^{1,3}Olayanju A.O*, ²Akanni E.O., ¹Akinseye J.F., ¹Akele R.Y., ³Eluke B.C., ¹Olayanju A. J and ⁴Enitan S.S.

¹Department of Medical Laboratory Science, College of Medicine and Health Sciences, AfeBabalola University, Ado Ekiti, PMB 5454, Nigeria

²Department of Medical Laboratory Science, Ladoké Akintola University of Technology, P.M.B. 4400, Osogbo, Osun State, Nigeria

³Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu State, Nigeria

³Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu State, Nigeria

ABSTRACT

Human platelet antibodies are often implicated in some disease conditions, such as neonatal alloimmune thrombocytopenia (NAIT), idiopathic thrombocytopenic purpura (ITP) and platelet refractoriness. There is paucity of information on the development and relevance of this antibody in pregnant black women, especially in Nigeria. This study was therefore designed to determine the occurrence of antiplatelet antibody in pregnant women. Eighty-nine (89) pregnant women attending a tertiary health facility in Osogbo participated in the study. Platelet count and human platelet antigens (HPA) were investigated using standard protocols of automation and ELISA respectively. Thirty-two (34%) of the women were thrombocytopenic and antiplatelet antibody was detected in 17 (53%) of this sub-set. The antibody was of higher frequency in multiparous women and more prevalent within the age group of 26-30, especially those with history of previous blood transfusion. The study shows the imperativeness of including the platelet quantitative and functional assays as part of routine antenatal testing and monitoring.

Keywords: pregnancy, immune thrombocytopenia, platelet antibodies

INTRODUCTION

Thrombocytopenia is one of the most common hematologic abnormalities during pregnancy only second to anemia [1]. It has been reported that Platelet count decreases in normal pregnancy possibly due to increased destruction and haemodilution with maximal decrease in the third trimester [2] increased platelet consumption, and increased level of thrombane [3].

The two most common types of thrombocytopenia in pregnancy are gestational thrombocytopenia (GT) and immune thrombocytopenia purpura (ITP). Other causes are preeclampsia, infections such as malaria, folate deficiency and diseases such as leukaemia and aplastic anaemia [4].

Gestational thrombocytopenia is self-limiting and resolves within 1 to 2 months after delivery [5].

ITP occurs in 1-2 in 1000 pregnancies, but is the most common cause of isolated thrombocytopenia in the first and early second trimesters [6].

In women with no history of ITP, platelets count below $100 \times 10^9/L$ early in pregnancy and declining as gestation progresses are more consistent with ITP than GT. The situation becomes more complicated if a low platelet count is detected during third trimester [7].

Whenever the platelet count is $<50 \times 10^9/L$ in the presence of obstetric complications, the diagnosis should be immune thrombocytopenia purpura by default.

Immune thrombocytopenia (ITP) formerly known as idiopathic thrombocytopenic purpura is a common hematologic disorder caused by evolution of auto-antibodies against platelets. It is mostly due to IgG antibodies (92%), others such as IgM and IgA antibodies are also implicated in few cases. These antibodies coat the platelets leading to their accelerated and premature destruction in the spleen[8].

Immune thrombocytopenia accounts for 3-5% of all thrombocytopenia gravidis. It is the most common cause of thrombocytopenia in the first and second trimester [5],[7],[9]. ITP is an autoimmune disorder caused by development of immunoglobulin G autoantibodies that are directed against several platelets glycoproteins [10]. Antibody bound platelets are rapidly cleared from maternal circulation once they bind to specific antibody receptors on macrophages, found mainly in the spleen and also in the liver [10]. These IgG antibodies can cross the placenta and have the potential to cause thrombocytopenia in the fetus [11]. It is hypothesized that in a typical African setting where the cultural beliefs encourage multiple pregnancies, the proportion of these antibodies could be high among multiparous women thus rendering them "dangerous blood donors".

Apart from the risk that these antibodies pose to recipients when these women are recruited as donors, their neonates may also possess neonatal alloimmune thrombocytopenia. The most serious complication of neonatal alloimmune thrombocytopenia is intracranial haemorrhage, which occurs in 10-20% of symptomatic infants [12][13][14][15]. Up to 80% of these bleed prenatally [16].

The study was designed to screen for the incidence of anti-platelet antibodies in pregnant black women to determine the extent to which immune mechanisms account for thrombocytopenia.

MATERIALS AND METHODS

Subjects' selection: Eighty nine consenting pregnant women who were registered at the antenatal clinic of LadokoAkintola University of Technology Teaching Hospital, Nigeria participated in the study. Among the pregnant women who gave informed consent, only those who were normotensive with blood pressure less than or equal to 140/ 90 mmHg and those without any history of haematologic disorder were included. Pregnant women on non-steroidal anti-inflammatory drugs such as aspirin; and on conditions such as Splenomegaly, Connective tissue disease such as SLE, hypertension, bleeding disorders, HIV and hepatitis B infection were excluded from the study.

A questionnaire consisting of subjects' demographic data, clinical history, other obstetrics details and consent form was administered to the participants. The subjects that met the inclusion criteria were recruited for the study and signed an informed consent form.

Ethical Clearance

Ethical clearance was sought and obtained from the ethical committee of LAUTECH teaching hospital Osogbo.

Blood Sampling

Blood specimen was withdrawn from ante-cubital vein using a dry sterile disposable syringe and needle. Five (5mls) of blood was collected from each subject and dispensed into ethylenediaminetetraacetic acid (EDTA) anticoagulant bottle. The specimens were labeled with subject's age, sex and identification number. The sample was kept at room temperature until processed for manual platelet count within two hours of collection. Plasma was separated from the tubes after centrifugation and kept frozen at $-20^{\circ}C$ for anti-platelet antibody ELISA.

Statistical analysis

Simple proportional analysis using Excel package was used.

Platelet Count

Principle: Ammonium oxalate lyses red cells and preserves platelets which are small refractile particles.

Procedure: One in 20 dilution of blood was made by 0.02mls of blood to 0.38mls of diluting fluid in a test tube, the tube was tightly covered and the content of the tube was mixed for 1minute, it was allowed to stand for 10minutes

for complete haemolysis of red blood cells. The cover slip was fixed on the counting chamber and charged with a pasteur pipette after thorough mixing, the chamber was placed in a petri dish containing a piece of moist filter paper and cover for 20minutes to allow the cells to settle and to avoid dryness, $\times 40$ objective was used to count the cells present in the 5 of 0.04mm area using Thoma's rule of cell counting and calculated as described by Dacie and Lewis [17].

Antiplatelet Antibody

APAB ELISA kit was used for the detection of antiplatelet antibody, the blood was centrifuged for 20min at 3000rpm and the plasma was carefully harvested and stored in aliquot at -20°C until use.

Principle: When a known antigen is attached to a solid phase (a micro titre well), it is reacted with the test sample which may contain antibody. The test is incubated and followed by a wash, an enzyme-labelled antihuman globulin is reacted with the antibody in the test sample that has attached to the antigen on the solid phase. After incubation, the uncombined labelled anti-human globulin is washed off. Retention of enzyme on the surface is detected by addition of substrate which changes colour after incubation. The reaction is stopped by changing the pH with an acid. The concentration of antibody in the test sample bound by antigen on the solid surface is proportional to the intensity of the colour.

Assay Procedure

Wash solution – One volume of wash solution was diluted in nineteen volumes of deionised water.

Standard wells, sample wells and blank (control) wells was set, 50 μl of standard was added to each standard well, 50 μl of sample was added to sample well, 50 μl of sample diluent was added to each blank /control well. 100 μl of horseradish peroxidase(HRP)-conjugate reagent was added to each well, cover with an adhesive strip and was incubated for 60 minutes at 37°C . The microtiter plate was washed with an automatic washer by delivering and aspirating 5 times. 50 μl of Chromogen solution A and 50 μl of Chromogen solution B was added to each well. It was gently mixed and be protected from light to incubate for 15 minutes at 37°C . 50 μl Stop solution was added to each well. The color in the well was change from blue to yellow. The optical density (O.D) was read at 450nm using micro-elisa strip plate reader with in 15minutes.

RESULTS

A total of eighty-nine(89) consenting pregnant women participated in this study, age range between 21- 40 years. 32 (36%) of this subjected were thrombocytopenic (as defined by a low platelet count of $<150 \times 10^9/\text{L}$). 17 (53%) of this thrombocytopenic subjects had circulating antiplatelet antibodies (APAb) as shown on table 1 and 2.

Table 2 also shows the age range distribution of APAb which revealed the ranges 26 -30 as the group with the highest seroprevalence of APAb at 69.2% followed by 31 – 35 which had a 50% prevalence.

Table 1: Prevalence of thrombocytopenia in pregnant participants.

Thrombocytopenia	Number(percentage)
Positive ($< 150 \times 10^9/\text{L}$)	32 (36%)
Negative ($> 150 \times 10^9/\text{L}$)	57 (64%)
Total sample	89 (100%)

Table 2: Age distribution of thrombocytopenic pregnant women who participated in the study in relation to the incidence of anti-platelet antibody (APAb)

Age	Total number	APAb positive (%)	APAb Negative (%)
21 – 25	9	4 (44.4)	5 (55.6)
26 – 30	13	9 (69.2)	4(30.8)
31 – 35	6	3 (50.0)	3 (50.0)
36 – 40	4	1 (25.0)	3 (75.0)
Total	32	17 (53.1)	15 (46.9)

DISCUSSION

Antibody formation against alloantigens of the human platelet membrane is responsible for clinical syndromes and transfusion related conditions such as neonatal alloimmunethrombocytopenia(NAIT), Post transfusion purpura (PTP), platelet transfusion refractoriness (PTR)[18].

This work was designed to determine the seroprevalence of antibodies to human platelet antigen (APAb) in thrombocytopenic pregnant women. In our study we detected antiplatelet antibodies in the serum of 17 out of 32 thrombocytopenic pregnant women, thus a seroprevalence of 53.1% as against 41.7% previously reported [19] among pregnant women in Rivers state Nigeria. The report attributed this to generation of antibodies against integrin adhesion molecules on the fetal platelets therefore antibodies develops if sufficient number of fetal platelet cross into maternal circulation, also repeated pregnancy can cause maternal antibodies to reach a level sufficient to cross into the fetal circulation where they impair platelet function and even evoke neonatal thrombocytopenia a condition known as neonatal alloimmune thrombocytopenia. The higher prevalence detected in our study may be due to a smaller sample size.

The highest seroprevalence of APAb was detected in the age range between 26 – 30 years which was at 69.2% followed by 31 – 35 years which amounted to 50% and the lowest being ages 36 – 40 which had 25% prevalence.

About eighty-three (83.3%) of subjects detected to be positive for APAb had history of blood transfusion and only 16.7% never had any history of blood transfusion. None of our APAb positive subject ever had a history of haematological disorder.

In this study, 90.5% of APAb seropositive subjects were individuals within two and below level of parity while the remaining 9.5% were individuals with three and above level of parity.

There were number of socio-demographic factors that were predictive for prevalence of antiplatelet antibodies population like age group, seropositive rate 9(69.2%) of Antiplatelet antibodies (APAb) has its highest prevalence among the thrombocytopenic pregnant women which fell within the ages of 26-30.

Previous studies suggested that alloantibodies against human platelet antigens are involved in neonatal alloimmune thrombocytopenia, post transfusion pupura and refractoriness to random donor platelet [20]. The present study outcome is similar with other studies in Caucasians[21], which suggest that the presence of antiplatelet antibodies in mothers and fetus during pregnancy is associated to platelet destruction and consequently thrombocytopenia. This often results in severe hemorrhagic diseases such as acute neonatal alloimmunethrombocypenicpupura, neonatal alloimmune thrombocytopenic pupura, post transfusion pupura and platelet refractivity,

This prevalence of antiplatelet antibodies in thrombocytopenic pregnant women (within the age group of 26 to 30 in this study was high. 36% of the pregnant women who were observed to have marginal decreases in platelet count were notably placed on haematinics.

Therefore, thrombocytopenic pregnant women should be screened for antiplatelet antibodies in order to prevent congenital abnormality such as intercranial hemorrhagein the foetus.

Conflict of interest

The authors hereby declare there is no conflict of interests associated with this study or any of the procedures and materials used for the purpose of the study.

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