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ORIGINAL ARTICLE

Platelet activities in pregnant women living with Human Immunodeficiency Virus on HAART in Lagos, Nigeria

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ABSTRACT

Introduction: Thrombocytopenia due to insufficient thromboxane A_2 activation has been an independent predictor associated with bleeding in some pregnant women living with HIV. More so, the elevation in the systemic vasoconstriction by the abnormal activation of platelets through thromboxane A_2 is a multi-system disorder during pregnancy resulting in maternal hypertension which is one of the major underlying pathophysiological occurrences in women with preeclampsia.

Materials and Method: A cross-sectional, descriptive study to determine platelet activities in pregnant women living with Human Immunodeficiency Virus on HAART residing in Lagos State, was carried out. A total of 80 samples were collected; 40 from pregnant women on HAART (group A), 20 samples from HIV negative pregnant women (group B) and 20 from non-pregnant HIV positive women on HAART (group C). The samples were analyzed using automated haematology analyzer (MIDRAY BC 10), platelets morphology was reviewed from blood films stained by Leishman stain and thromboxane A_2 was quantified using standard ELISA technique. Data was analyzed with SPSS version 23.0. Values were considered significantly different at $P < 0.05$.

Results: The platelets count results revealed the mean ± standard error of mean (SEM) in group A, group B and group C as 236.27 ± 11.40, 227.26 ± 16.44 and 246.21 ± 21.54 respectively (p = 0.743). The Platelet distribution width (PDW) revealed the mean ± SEM in group A, group B and group C as 13.50 ± 0.42 ,13.36 ± 0.53 and 12.56 ± 0.52 (p = 0.390) . As for mean platelet volume (MPV), the mean ± SEM for group A,

group B and group C were 12.36 ± 1.96, 10.71 ± 0.37, and 10.45 ± 1.78 respectively(p = 0.671). The mean ± SEM of thromboxane A_2 in group A, group B and group C were 367.65 ± 37.74, 135.85 ± 51.69 and 276.10 ± 50.90 (p = 0.02).

Conclusion and Recommendation: This study showed platelet aggregation in group A, group B and group C were 7.5% , 0%, and 2%; and this could be one of the suggestive factors in the increase in coagulation activities found in pregnancy. Thromboxane A_2 level reflects a recent activation of platelets, thus it serves as a good biomarker. It is recommended that research be conducted to establish reference ranges of Thromboxane A_2 for categories of normal individuals, including pregnant women in Lagos while attention should be paid to platelet activity studies in the Prevention of Mother to Child Transmission (PMTCT) programmes, to prevent mortality of participants on account of platelet aberrations.

Keywords: HIV, HAART, PMTCT, Platelet Distribution Width, Thromboxane A2

INTRODUCTION

Most women in developing countries like Nigeria experience severe or other life threatening health issues during pregnancy or childbirth. These complications during pregnancy and childbirth resulted to an increase in mortality and morbidity compared to other reproductive health problems in African countries generally.

The rate of maternal mortality in Nigeria between 1999 and 2015 is among the highest in the world ranking the 10th out of 183 countries. The maternal mortality ratio is 1:100, with an estimated 59,000 annual maternal deaths making Nigeria the leading contributor to the maternal death in Africa.

The prevalence HIV infection in Nigeria is around 3.6% which is third largest number of HIV infected people in the world. According to the report released by the Government of Nigeria, the national HIV prevalence in Nigeria is 1.4% among adults aged 15–49 years. Previous estimates had indicated a national HIV prevalence of 2.8%. In spite of Nigeria's national HIV prevalence rate of 1.4% among adults aged 15–49 years, women aged 15–49 years (1.9%) are more than twice as likely to be living with HIV than men (0.9%). The difference in HIV prevalence between women and men is greatest among younger adults, with young women aged 20–24 years more than three times as likely to be living with HIV as young men in the same age group . The epidemic of HIV in Nigeria had shown that pregnant women are more vulnerable and adversely affected due to the compromised state of their immunity.

Many studies have shown that pregnant women living with HIV have between 2–10 times increase risk of mortality than uninfected

pregnant women[7]. There is a strong evidence that there is more risk in pregnant women in low-income countries in sub-Saharan Africa with significantly higher probability of maternal and child deaths.

The platelets multifunctional roles can also be associated with maternal and infant mortality rates. Thrombocytopenia due to insufficient thromboxane A_2 activation has been an independent predictor associated with bleeding in pregnant women living with HIV .

The pathway of platelets production during haematopoiesis in a sub-process is called thromopoiesis. Thrombopoiesis takes place in common myeloid progenitor cells in the bone marrow, starting with megakaryoblast, which first differentiate into promegakaryocytes and then into megakaryocytes. Megakaryocyte and platelet production is regulated by a hormone called thrombopoietin, which is produced by the liver and kidneys, and the process operates a feedback mechanism. The protoplatelets then proliferates into hundreds of platelets that circulate in the bloodstream, while the remaining nucleus of the ruptured megakaryocyte is consumed by macrophages. Each megakaryocyte produces between 5,000 and 10,000 platelets before its cellular components are fully depleted. Altogether, around 10^{11} platelets are produced each day in a healthy adult. The average lifespan of a platelet is just 5 to 10 days[7] . The destruction of old platelets by macrophage phagocytosis takes place in the spleen and by Kupffer cells in the liver. Large volume of platelets are stored in the spleen as a reserve, which is released on feedback by sympathetically-induced splenic muscle contractions during severe injury . Thromboxane A_2 (TxA_2) belongs to the family of lipids known as eicosanoids, which are metabolites of arachidonic acid. They are produced by the sequential action of three enzymes namely; phospholipase A_2, Cyclooxygenase (COX) and TxA_2 Synthase (TXAS). TxA_2 is described as being majory released from platelets activities, with some minute secretions from endothelial cells, macrophages and neutrophils . Thromboxane A_2(TXA_2) is known to be a potent stimulator of platelet activation, aggregation, and vascular constriction. Thromboxane A_2 exhibits its pathophysiological activity by binding to a G-protein-coupled specific receptor, the thromboxane prostanoid (TP) receptor . TXA_2 may also act as a potent angiogenic stimulator directly as well as indirectly by supplying cytokines such as pro-angiogenic factors upon platelet aggregation. TXA_2 may enhance angiogenesis by enhancing the interactions between platelets and endothelial cells . In HIV-infected individuals, there is a direct HIV binding and interactions with platelets leading to platelet hyper-activation, micro-particle formation, platelet reactivity and aggregation to the blood vessels, immune cells and also erythrocytes .The aim of this study was to determine platelet activities in pregnant women living with HIV on HAART in Lagos State.

Materials and Methods

Study Design

This was a cross-sectional, descriptive study of HIV positive pregnant women on HAART attending ante natal clinics in Tertiary health facilities in Lagos State.

Study Area

Geographically, Lagos State is lying approximately on longitude 3° 23' 40.81"E and latitude 6° 27' 14.65"N, located on the South–Western part of Nigeria .The population of Lagos was estimated to be over 21 million in 2016 and is the largest city of Africa.

Duration of Study

This study was conducted between November 2022 and February 2023.

Sample Size
A total of 80 samples from three different groups, viz: 40 pregnant women on prevention of mother to child transmission (PMTCT), 20 HIV negative pregnant women and 20 HIV positive women (not pregnant) of child bearing were recruited.

Sample Collection
A volume of 5mls of sample was collected into Ethylenediamine tetraacetic acid (EDTA) bottle from each participant.

Laboratory Procedure
Each sample collected was transported immediately to the College of Medicine, Lagos State University, O&G Research Laboratory Department (LASUCOM-LASUTH) under one hour of collection, without exposing the sample to cold or extreme heat. Full blood count was done immediately with auto analyzer BC-10. The samples were then spun at 1000rpm for 15minutes according to manufacturer's instruction to harvest plasma samples which were stored at -20°c until used for Thromboxane A_2 Assay. Thromboxane A_2 was assayed for, using ELISA technique in which polystyrene microwells pre-coated with monoclonal antibodies specific to Thromboxane A_2 was employed. Participant's plasma sample was added to the microwell together with a second antibody conjugated enzyme and directed against the Thromboxane A_2. During incubation, the specific complex formed was captured on the solid phase. After washing to remove sample plasma proteins and unbound HRP-conjugate antibody, chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide were added to the wells. In presence of the antibody-antigen-antibody "sandwich" immuno-complex, the colorless TMB Solution are hydrolyzed by the bound HRP-conjugate antibody to a blue-colored product. The blue color turns yellow after stopping the reaction with acid. The amount of color intensity was measured spectrophotometrically with ELISA plate reader and it is proportional to the amount of Thromboxane A2 captured in the wells, and to its amount in the sample respectively.

Data Analysis: Data was analyzed using SPSS 23.0 statistical package where ANOVA was used as the tool for comparison. P-value of < 0.05 was considered as evidence of significant statistical difference.

Results
The correlations of Thromboxane A2 in women on PMTCT with Platelet, PDW and Age (-0.049, 0.061, and 0.098) were not significant ($P > 0.05$). Details are as depicted in Table 1. The correlation of Thromboxane A_2 with Platelet, PDW and Age were equally insignificant in non-pregnant women on HAART and HIV negative women ($P > 0.05$). Details of the correlation coefficients and p valus are depicted in Tables 2 and 3.

However, thromboxane A_2 are significantly elevated in pregnant women on HAART (367.65±37.74pg/mL) and relatively elevated in non-pregnant women on HAART too (276.10±50.90pg/mL) as against HIV-negative subjects where 135.85±51.69pg/mL was recorded (p=0.02). The details of inter-groups comparison with ANOVA is presented in Table 4.

Table 1. Correlation of Thromboxane A_2 in HIV positive pregnant women on HAART with other platelet parameters and age.

	r	p
Thrombo xane / PLT	- 0.049	0.766
Thrombo xane / PDW	0.061	0.709
Thrombo xane / AGE	0.098	0.548

r = Pearson correlation coefficient * P significant @ p <0.05
- r value =Inverse relationship + r value =Direct relationship

Table 2. Correlation of Thromboxane A_2 in HIV negative pregnant women with other platelet parameters and age.

	r	p
Thromboxane / PLT	- 0.151	0.525
Thromboxane / PDW	- 0.028	0.908
Thromboxane / AGE	- 0.130	0.586

r =Pearson correlation coefficient * P significant @ P < 0.05
- r value =Inverse relationship + r value =Direct relationship

Table 2. Correlation of Thromboxane A_2 in HIV negative pregnant women with other platelet parameters and age.

	r	p
Thrombo xane / PLT	- 0.151	0.525
Thrombo xane / PDW	- 0.028	0.908
Thrombo xane / AGE	- 0.130	0.586

r =Pearson correlation coefficient * P significant @ P < 0.05
- r value =Inverse relationship + r value =Direct relationship

Table 3. Correlation of Thromboxane A_2 in HIV positive, non-pregnantwomen on HARRT with other platelet parameters and age.

	r	p
Thrombo xane / PLT	0.116	0.626
Thrombo xane / PDW	0.233	0.322
Thrombo xane / AGE	0.108	0.651

r =Pearson correlation coefficient * P significant @ P < 0.05
- r value =Inverse relationship + r value =Direct relationship

Table 4. Comparison of the platelet parameters across the groups (ANOVA)

	Group A	Group B	Group C	f	p
PLT (10^9/L)	236.27±11.40	227.26±16.44	246.96±21.54	0.311	0.943
PDW (%)	13.50±0.42	13.36±0.53	12.56±0.52	0.952	0.390
TXA2(pg/mL)	367.65±37.74	135.85±51.69	276.10±50.90	6.557	0.02
Age(years)	32 .65±0.72	31.30±0.84	33.25±1.23	0.938	0.396

P signifi cant @ P < 0. 05

Discussion

Several studies on platelet activities had been conducted .This study had shown that there is no significant statistically difference in the mean of platelet count in pregnant women in cases (group A) and controls (group B and group C) with a reference range of 100 x10^9/L - 400 x10^9/L .This in line with the study conducted by Babah *et al* in a cross sectional at University of Lagos Teaching Hospital (LUTH) with the mean of 205.48 x10^9/L in pregnant women and 234.75 x10^9/L in non pregnant women[19] . The study had shown no significant difference in PDW which was attributed to blood volume expansion that occurs during pregnancy and suggested that the increase in PDW in pregnancy might contribute slightly to the hypercoagulability associated with pregnancy . In the study reported by Tesfay et al,[20] there was an increase in mean platelet count in the pregnant women compared to the non-pregnant group (226.54 x 10^9/L versus 214.95 x 10^9/L) but no significant difference in the PDW ($p > 0.05$) . A significant increase in the concentration of thromboxane A_2 was observed in HIV positive pregnant women on HAART with a reference range of 292.17pg/mL - 443.13pgpg/mL compared to HIV negative women and non-pregnant HIV positive women (P= 0.02) . This may be attributed to hyper-responsiveness of platelets activities in pregnant women on HAART.

CONCLUSION

This study had shown thromboxane A_2 to be a significant biomarker of platelet activities. Platelet activities are multi-factorial in nature. Thromboxane A2 reflects the recent activation of platelet which gives signals of potential haemorrhage. The action of thromboxane A_2 in autocrine and paracrine activities generate more thromboxane A_2 amplification as a platelet agonist. Thus, thromboxane A_2 is a potent platelet activator and vasoconstrictor that may have pathological consequences when activation is not balanced or controlled. Platelet activities of women on HAART should be studied, to access the propensity to bleeding, especially during pregnancy

CONFLICT OF INTERESTS

There is no conflict of interests.

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