Comparative Study of Fibrinolytic Response amongst Malarious Pregnant and Non Malarious Subjects in Rivers State, Nigeria

Stella Urekweru Ken-Ezihuo1*, Barinaaziga Sunday Mbeera1, Chiatugu Nancy Ibeh2 and Zacchaeus Awortu Jeremiah3

1Department of Medical Laboratory Science, Rivers State University, P.M.B. 5080, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.
2Department of Medical Laboratory Science, Nnamdi Azikiwe University, Akwa, Anambra State, Nigeria.
3Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Authors’ contributions
This work was carried out in collaboration among all authors. Author KESU designed the study and performed the statistical analysis. Authors JZA and INC approved and supervised the study. Authors KESU, JZA and INC managed the analyses of the study. Authors KESU and MBS managed the literature searches. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/IBRR/2020/v11i230128

ABSTRACT

Aim: The study was designed to comparatively assess the degree of fibrinolytic response amongst malaria-positive pregnant women, and non-malaria positive subjects in Rivers State, Nigeria.

Methods: The study area covered University of Port Harcourt Teaching Hospital, Port Harcourt [UPTH] and Rivers State University Teaching Hospital, [RSUTH] both in Port Harcourt metropolis Rivers State. It was a cross-sectional study carried out on a total of two hundred and forty female attendees at the obstetrics and gynecology clinics of the two hospitals. The subjects were grouped into three comprising of eighty subjects in each group; malarious pregnant women, non-malarious pregnant women and apparently healthy non-pregnant women. Venous blood sample measuring 5 milliliter volume was drawn from each subject. The sample was dispensed into two separate EDTA anticoagulant bottles, 3 milliliter and 2 milliliter meant for measuring the levels of markers of

*Corresponding author: E-mail: stellakenezhuo@yahoo.com;
Keywords: Fibrinolytic markers; malaria-positive; pregnancy; malaria infection.

1. INTRODUCTION

Malaria infection has been described as a pathological condition associated with significant mortality and morbidity [1]. In the areas of Africa where malaria is endemic, an average prevalence value of 27.8% representing 1.4 of the population of pregnant women are infected with malaria parasitaemia [2]. It is estimated that 125 million pregnant women are living in malaria predominant areas, and 32 million pregnancies are at risk of complications arising from malaria infection [3]. Some of the complications according to [2] could be either maternal or fetal which include; miscarriages, still birth, preterm labour and delivery, anaemia among others. In Nigeria, major cases of maternal and new born mortality have been associated to malaria infection [4]. The Nigeria federal Ministry of Health in 2007, stated that 11% of pregnant women die as a result of malaria [5].

Previous studies show that during malaria infection as well as during pregnancy, the overall fibrinolytic activity of the haemostatic system is impaired as compensation to the impaired coagulation [6]. Pregnant women usually, develop physiological haemostatic system switch which serves for the maintenance of placental function to accommodate the new developing fetus. This is an outcome of intense changes taking place in the coagulation and fibrinolytic activities often observed in pregnancy. Also a direct attack of the malaria parasite launched on the endothelium of the microcirculation tear down the endothelial cell leading to the release of varying kinds of cytokine and inflammatory intermediaries by the endothelium and some other cells involved in the harboring of these inflammatory markers [7].

Since the third component of the sustainable Development Goals [SDGs] by United Nations after 2015 is targeted at reducing global maternal mortality ratio to less than 70% per 100,000 live births by 2030 [8], it became necessary to investigate and compare the concentrations of markers of fibrinolysis amongst malaria-positive pregnant women, normal pregnant and apparently healthy non-pregnant women, to determine the impact of malaria on the markers. This will help in the management of the exacerbating clinical conditions such as thromboembolism, fetal growth restriction, miscarriage, and maternal mortality and morbidity which may arise in pregnancy complicated with malaria, especially in Nigeria, where in spite of the huge human and financial commitment involved in the stoppage of malaria and reversal of its incidence, the first and sixth Millennium Development Goals [MDGs] could not achieve the set goals.

2. MATERIALS AND METHODS

2.1 Study Design

The cross sectional study was designed to involve pregnant and non pregnant subjects.
subjects were recruited after giving consent by providing the information on the well structured questionnaire given to them to capture relevant data which included their demographic and clinical information such as age, on anti-malaria or malaria vaccine, anti-inflammatory drugs, human immune-deficiency virus [HIV] drugs, cancer drugs or hepatitis B surface antigen [HBsAg], hepatitis C virus [HCV] drugs. The subjects who did not give consent were excluded.

2.2 Study Area

The study involved two tertiary hospitals, the University of Port Harcourt Teaching Hospital, Port Harcourt [UPTH] and Rivers State University Teaching Hospital, [RSUTH]. The two hospitals are located in Port Harcourt, capital city of Rivers State, Nigeria. They have the several units usually found in tertiary hospitals, which include; well managed ante-natal care clinics.

2.3 Study Population

Participants were recruited from among attendees of the Obstetrics and Gynecology department of the two tertiary hospitals in Port Harcourt metropolis. They comprised of eighty malaria-positive pregnant women, eighty non-malaria positive pregnant women and eighty apparently healthy non-pregnant women making a total of two hundred and forty participants selected by the stratified random technique. All subjects were within the age of 18-50 years.

2.4 Blood Sample Collection and Storage

Whole blood sample of five milliliters volume was collected from each subject into EDTA [ethylene diamine tetra acetic acid] bottles using standard venepuncture technique described by [9]. One part of the sample measuring three milliliters was spun at 1000rpm for 15-minutes, and using a clean pasture pipette, the plasma supernatant was transferred into plain vials and stored in the refrigerator at -20°C, kept until the last day of sample collection which took 21 days. The stored plasma sample was later used for the assay of markers of fibrinolysis [Plasminogen, Plasminogen activator inhibitor-1 [PAI-1], Plasminogen activator inhibitor-2 [PAI-1], Tissue plasminogen activator [t-PA], alpha-2-antiplasmin, D-dimers and fibrinogen], the other part of the sample dropped in the second EDTA bottle was used for diagnosis of malaria and pregnancy test.

2.5 Assay of Fibrinolysis Markers

Concentration of the markers of fibrinolysis was estimated using sandwich Enzyme linked immunosorbent assay [ELISA] method described by [10]. All reagents used were provided in the kits by the manufacturer [Elabscience Biotechnology Inc. Company, China]. A total of 96 microplates ELISA wells were used to conduct the assay in phases. At the first phase, 100 μl of diluent was added into the 96 wells, 100 μl of the different concentrations of the standard was added side by side into each of the 16 wells on first two columns. 100 μl of samples were then added into the remaining 80 wells. After addition of diluent, standards and samples, the microplate wells were then incubated at 37°C for 90 minutes.

At the second phase, diluent plus sample and diluent plus standards were decanted, and 100 μl of the biotinylated detection antibody working solution was added into each of the 96 wells. These wells were again covered with the microplate sealer, mixed gently and incubated at 37°C for 60 minutes before the solution was decanted. Third phase started with addition of 350 μl of wash buffer into each of the wells, soaked for 2 minutes and decanted. The microplate wells were then turned upside down against clean absorbent paper to pat dry. This process was repeated for 3 times.

At the fourth phase, after all the washings, 100 μl of the Avidine-Horseradish Peroxidase [HRP] Conjugate working solution was added to each well. The wells were then covered with the plate sealer and incubated for 30 minutes at 37°C. After the incubation the solution was decanted from each of the wells and then 350 μl of wash buffer was added to each of wells and soaked for 2 minutes as a process of washing the wells again. This process was repeated for three times as conducted in step 3.

The fifth phase involved the addition of 90 μL of substrate reagent into each of the wells, after the addition it was covered with a new plate sealer and incubated for about 15 min at 37°C. At this stage the plate was protected from direct light and the reaction time was monitored closely. According to the actual colour change in the reaction, the time was either shortened or extended by adding into the wells, 50 μl of the stop solution provided in the kit, but the time was not more than 30 minutes. After addition of the stop solution, the optical density [OD value] of
each well was determined at once with a microplate reader set to 450 nm. The results were extrapolated from the calibration curve by comparing optical density of samples with the standard curve.

2.6 Diagnosis of Malaria

Blood films were prepared from each blood sample on clean dried slides. The smears were air dried and stained with 3% Giemsa for parasite identification as described by [11]. A positive smear was included with each new batch of working Giemsa stain for quality control. After drying, the presence and identification of malaria parasites was performed by a trained and experienced microscopist.

2.7 Pregnancy Test

The Consult Diagnostics hCG Combo Test was used. It is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin [hCG] in urine or serum to aid in the early detection of pregnancy. The assay was conducted by adding plasma into the specimen well in the test cassette followed by the addition of colored antibody conjugates and observing the formation of colored lines as positive. To serve as a procedural control, a colored line will always appear at the control line region if the test has been performed properly.

2.8 Statistical Analysis

Statistically Analysis System [SAS] version 9.4 was used to analyze the data. The results were expressed as mean± SEM. One-way analysis of variance [ANOVA] was used for comparison of mean differences in the various groups, followed by Test of association which was performed using Pearsons’ correlation with results being represented in box plots to show nature of association. Level of significance was set at P<0.05.

2.9 Eligibility Criteria

All subjects in the study were females within the age of 18-50 years. Subjects whose pregnancy test were positive and they also tested positive for malaria parasitaemia. Pregnant subjects who tested negative for malaria parasitaemia. Apparently healthy non pregnant subjects who were negative for pregnancy and malaria parasitaemia were included. All pregnant and non pregnant subjects who were currently malaria treatment or malaria vaccine, those who had evidence of diseases such as neoplasm which could cause elevated level of hCG, and subjects who refused to give informed consent were excluded from the study.

3. RESULTS

3.1 Concentrations of the Fibrinolytic Markers in the Different Study Groups

The concentrations of fibrinolytic markers [Fibrinogen, Tissue plasminogen activator, D-dimer, Plasminogen activator inhibitor-1, Plasminogen activator inhibitor-2, Plasminogen and Alpha-2 antiplasmin] of the study groups are shown in Table 1. The mean ± SEM of fibrinogen in malaria infected pregnant women; 760.44±16.18 ng/ml was elevated when compared with the non pregnant control values of 704.73±15.25 ng/ml. Whereas, the concentration of the non infected pregnant women; 697.70±18.84 ng/ml was observed to be lower compared to the non pregnant control value of 704.73±15.25 ng/ml. These values were significantly different between the Study groups [P<0.01]. The fibrinogen concentration in the non-pregnant women was significantly higher than that of the malaria negative women [P<0.05]. However, infection with malaria resulted in a significant rise in the level of fibrinogen 697.70±18.84 ng/ml in malaria negative women to 760.44±16.18 ng/ml in the malaria-positive pregnant women [P<0.05].

The concentration of Tissue plasminogen activator [tPA] in the malaria-infected pregnant women, non infected pregnant women and non pregnant control subjects were, 46.39±2.69 ng/ml, 28.87±1.39 ng/ml and 31.34±1.64 ng/ml respectively. The variations were significantly different [P<0.01]. Whereas the level of tPA was seen to reduce from 31.3±1.64ng/ml in the non-pregnant to 28.87±1.39 ng/ml in the non-infected pregnant women, it was observed that the presence of malaria caused an increase to 46.39±2.69 ng/ml in level of tPA in the malaria-infected pregnant women. [P<0.05].

Malaria infection was also seen to exert significant influence on the D-dimer values in this study. The D-dimer concentration value was lowest in the non pregnant control [30.24±1.04 ng/ml], followed by the non-infected pregnant women value [53.90±1.18 ng/ml] and malaria infected pregnant women [77.64±6.9 ng/ml]. The variation in the concentration of D-dimer was significantly different from each other between...
showed that the mean± SEM of α-2 antiplasmin in the women studied increased significantly from 1016.96±24.51 ng/ml in the non pregnant control to 1130.61±29.74 and 1314.06±34.64 ng/ml for the pregnant negative and pregnant positive women respectively [P<.001]. Like some other fibrinolytic markers, α-2 antiplasmin was seen to increase due to the presence of malaria parasite.

### 3.2 Boxplot Graphs of Fibrinolytic Markers among the Three Experimental Groups

Fig. 1 shows box plot graphs of fibrinogen among the three groups of subjects. Fibrinogen concentration was elevated to a significant level only among malaria positive pregnant women. This delineates malaria as a cause of increase in fibrinogen concentration.

Fig. 2 shows the trend in tPA values among the three groups of subjects. There was no difference between the tPA level of non-pregnant [control] women and malaria negative pregnant women. A noticeable significant level of increase occurred only in malaria positive pregnant women.

Fig. 3 shows the trend in D-Dimers values among the three groups of subjects. There was an elevation of D-Dimer values contributed by pregnancy in the malaria negative pregnant women.

In Fig. 4, a more pronounced increased was observed in the malaria positive pregnant group.

### Table 1. Concentration of fibrinolytic markers among the study groups (Mean ± SEM)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pregnant-positive (n=80)</th>
<th>Pregnant-negative (n=80)</th>
<th>Non-pregnant (control) (n=80)</th>
<th>Test statistics P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (ng/ml)</td>
<td>760.44±16.18</td>
<td>697.70±18.84</td>
<td>704.73±15.25</td>
<td>0.011**</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>46.39±2.69</td>
<td>28.87±1.38</td>
<td>31.34±1.64</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>D-dimer (ng/ml)</td>
<td>77.64±6.94</td>
<td>53.90±1.18</td>
<td>30.24±1.04</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>PAI-I (ng/ml)</td>
<td>89.73±2.14</td>
<td>80.00±1.81</td>
<td>65.47±2.33</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>PAI-2 (ng/ml)</td>
<td>568.00±12.51</td>
<td>456.31±5.94</td>
<td>427.86±6.95</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Plasminogen (ng/ml)</td>
<td>23.82±7.5</td>
<td>16.63±0.67</td>
<td>18.49±1.04</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>α-2-Antiplasmin (ng/ml)</td>
<td>1314.06±34.64</td>
<td>1130.61±29.74</td>
<td>1016.98±24.51</td>
<td>&lt;0.0001****</td>
</tr>
</tbody>
</table>

SEM: Standard error of mean; * Women were pregnant and positive for Malaria parasites; Women were pregnant but negative for Malaria parasites. Within each parameter, means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: **=p<0.01; ***=p<0.001; ****=p<0.0001; ns=Not Significant (p>0.05)
A similar trend was observed in PAI-1 concentration.

Fig. 5 Shows that the PAI-2 concentration was significantly increased in malaria positive pregnant women.

The trend is the same in plasminogen concentration as shown in Fig. 6 as well as alpha-2 antiplasmin as shown in Fig. 7.

The summary of the trends in fibrinolytic parameters revealed that malaria is a major contributor to derangements in fibrinolytic parameters in pregnancy.

4. DISCUSSION

Data gotten from the study revealed significantly elevated fibrinogen concentration among the malaria-positive pregnant women compared to the non-pregnant control women. Comparing the two pregnant groups, it was further observed that there was significantly elevated level of fibrinogen in the malaria-positive women compared to the pregnant women who were malaria negative. The elevated fibrinogen concentration among the malaria positive pregnant women is in consonance with the findings of [11,12,13] who reported elevated fibrinogen level in P. falciparum infection in normal subjects and in infected placenta in pregnancy resulting to poor birth outcome. The elevation in fibrinogen concentration in this study therefore could be the outcome of combined associated physiological and pathological changes caused by pregnancy and malaria respectively. According to [14], physiological or pathological changes usually result from haematological and haemostatic alteration whenever the situation arises, the deleterious effect often occurring; present a clinical episode that is more acute in malaria cases combined with pregnancy thereby leading to higher mortality than in non pregnant women. The increase in the level of fibrinogen in the malaria infected women could also be related to the secondary fibrinolysis occurring due to a mechanical disorder, medical or other causes, that may lead to bleeding [15].

The contrasting level of fibrinogen observed between the malaria-negative pregnant women and the non pregnant control was not obviously, a low concentration below pregnancy level, rather, the level of fibrinogen in the negative pregnant women was still within the normal range of pregnant level for fibrinogen which is 400 – 650 m/gf [16]. However, the raised value in the control subjects could be of concern in this study. After reviewing the result observed in this study among the non pregnant control subjects, it was noticed that they were all students and according to previous studies by [17], elevated plasma fibrinogen in normal subjects is associated with some risk factors like smoking, use of oral contraceptive, stress (mental and psychological stressors) and host of other factors. [18], also enumerated in their report that stress, use of oral contraception, advance age and other factors could be responsible for elevated fibrinogen in normal individuals. Looking from this perspective, it was suggested that the increase in the fibrinogen elevation in the control subjects could be due to high social class life style since they were all students who could be living lives the way chose to. It was thought that some of them could be on oral contraception as a way of preventing unwanted pregnancy, some could be smokers, some could be going through a form of stress or according to [19], and some could also be in the early days of their menstrual cycle. In a study by [19], they analyzed plasma fibrinogen in relation to estrogen and reported that fibrinogen level in early follicular phase is usually significantly elevated suggesting that in early follicular phase ovarian hormonal activity influences blood flow. The mechanism responsible for these remains unclear but theoretically, there may be increased risk of thromboembolism. Whereas the non infected pregnant women in this study had fibrinogen level slightly lower than the level seen in the non pregnant subjects, it could be thought that they are less likely to be of the high social class, they were not involved in any form of life style activities which could predispose them to a condition of risks factors which could result to excessive accumulation of blood clots in their body compared to the students control subjects. Therefore, considering these factors, the increase in fibrinogen in normal subjects is in concurrence with these risks factors being responsible for the elevation in non pregnant control subjects who were students.
Malaria parasite exerted significant influence on most of the fibrinolytic markers in the pregnant women. Tissue plasminogen activator [tPA] in malaria infected pregnant women was seen to be of significantly high level compared to the non pregnant control value. Our finding is at variance with previous reports of [20] and [21] where tPA level was low in malaria. But this finding in our study is similar to the previous report [22] and [23]. Whose studies reported reduced tPA values in pregnancy. However our finding differs from the perspective that reduced tPA occur as a result of increase in the plasminogen activator inhibitors during pregnancy. This study disagrees with this point because both PAI-1 and PAI-2 were seen to also increase at the same time with tPA during our study, but agrees with the report of [24] that between 20 weeks of pregnancy and...
term, PAI-1 and PAI-2 levels increase to three folds their normal levels and also a less dramatic increase in both u-PA and t-PA levels also are observed. The elevated value seen in the malaria infected pregnant women showed that, in malaria combined with pregnancy in this study, tPA is up regulated and it took the same pattern as was seen in fibrinogen level in malaria infected women. Also the observed values in the non infected pregnant women compared to the non pregnant control obviously took the same pattern that was similar to our observation in fibrinogen concentration among the experimental groups.

Fig. 3. Boxplot of D-dimer [ng/ml] by study group. [There is malaria induced elevation of D-Dimer levels]

Fig. 4. Boxplot of PAI-1[ng/ml] by Study Group. PAI-1 concentration is more elevated in malaria infected pregnant women
Fig. 5. Boxplot of PAI-2 [ng/ml] by study group. Malaria causes an increase in PAI-2 levels

Fig. 6. Boxplot of plasminogen [ng/ml] by study group. Plasminogen levels are increased in malaria infected pregnant women

This could be a confirmation of the report documented by [25] which stated that elevated tPA could be associated to anticipated thromboembolism recorded theoretically in the study of coagulation and fibrinolysis in pregnancy, hence it was said that tPA levels changes in parallel with fibrinogen in pregnancy in the direction of hypercoagulability. By this statement it could be inferred in this study that tPA levels changed in the same direction as fibrinogen levels. The up-regulation in tPA value of malaria infected pregnant women in this study could probably be due to the coexistence of malaria and pregnancy because [3] reported that malaria infection in pregnancy impairs trophoblastic invasion with the placenta leading to vascular dysfunction and subsequent growth restriction, such that the resulting inflammatory process and release of cytokines with the placenta also affects the functional ability of the placenta. It should be of note that these cytokines which are the interleukin 6 and Tissue Necrotic Factor [TNF] are responsible for inducing the production of anti-platelet and tissue plasminogen activator [tPA] in cases of coexistence of malaria with another condition that could lead to bleeding [3].
PAI-1 and PAI-2 were elevated in all the pregnant women though more elevated in malaria infected pregnant women than the other experimental groups. The observed elevation in all the pregnant women is in consonance with previous reports by [26] that plasminogen activators are elevated for the good of pregnant women and in gestational tissues as they are required for normal placentation especially the PAI-2. This result also agrees with the report of [27] which reported that increase in the concentration of PAI-1 and PAI-2 in malaria infected women could also be as result of large numbers of proinflammatory cytokines being released due to malaria infection as well as in pregnancy more over PAI-2 also increases as the pregnancy increases [27].

Malaria and pregnancy in this study influenced the value of D-dimer both in malaria positive pregnant and in malaria negative pregnant women respectively. The elevated D-dimer concentration in all the pregnant women is in agreement with reports of [28] and [23]. They recorded elevated D-dimer during pregnancy above their non pregnant controls. Similarly there was elevated D-dimer concentration found among the malaria positive pregnant women in this study compared to the pregnant-negative women and non pregnant counterparts. This finding agrees with the report by [3] and [12] who reported increase in D-dimer concentration in cases of Plasmodium falciparum during pregnancy and in P.falciparum infected patient respectively. Our findings about elevated D-dimer in pregnancy and in pregnant malaria positive in this study could be linked to D-dimer originating from fibrin and reflecting its synthesis and degradation hence fibrinogen also was observed to be elevated in this study. Despite the elevated levels of PAI-1 and PAI-2 during pregnancy in this study, a highly significant D-dimer level was still observed among the pregnant women, this could be happening as a marker of thrombosis generally. Also the elevated D-dimer may have occurred as a compensatory mechanism secondary to increase fibrin formation. Because D-dimer serves as a sensitive and specific in vivo marker on intravascular fibrin formation an indication that fibrin has been formed and digested intravascularly by plasmin in patients infected with falciparum.

Alpha-2 antiplasmin [α-2antiplasmin] was also raised in malaria positive pregnant women more than the negative pregnant women and the non pregnant control women. The elevated level in pregnancy generally agrees with the report of [29] and [30]. They both reported elevated level of α -2 antiplasmin in pregnancy. The higher level observed in malaria infected pregnant women could be attributed to complication of malaria in pregnancy leading to over production of all the biomarkers of fibrinolysis which is suggested to be likely due to the endothelial disturbances which may have compromised the endothelial cell function leading to a favorable formation of thrombus in malaria during pregnancy condition.
The increase in the level of plasminogen correlates with the reports of [30] and [31] who recorded increase in plasminogen during pregnancy. But the mechanism of plasminogen activation in vivo is poorly understood. However it has been documented that in impaired plaque formation seen in atherosclerosis-prone mice, plasminogen deficiency was observed. The increase in plasminogen in this study could be linked to the synergistic effect between coagulation and fibrinolysis in pregnancy combined with malaria.

5. CONCLUSION
Malaria was seen to exert significant impact on most of the fibrinolytic markers in the malaria positive pregnant women in this study. The levels of fibrinogen and tPA were seen to change in parallel direction towards hypercoagulability. The elevated D-dimer in this condition when compared to the non pregnant subjects is an indicator of thrombosis.

The high level of fibrinolytic markers observed in the pregnant women positive with malaria in this study was, therefore, concluded to be associated with compromised endothelial cell function which might have led to complications of over production of biomarkers of fibrinolysis. The outcome could be formation of thrombus and excessive bleeding in pregnancy leading to miscarriages, fetal death or maternal mortality.

CONSENT
Subjects recruited into the study were given a standard written informed consent form to fill before their blood sample was collected.

ETHICAL APPROVAL
The Research Ethics Committees of the University of Port Harcourt Teaching Hospital [UPTH] and that of Rivers State Hospitals Management Board gave the ethical approval for the study.

ACKNOWLEDGEMENT
Our gratitude goes to the Nurses at the department of Obstetrics & Gynaecology who assisted during subject recruitment and blood collection. We also appreciate all the staff of the department of Medical Laboratory Science for devoting their time to assist during the sample collection, preparation, storage and analysis. The two categories of staff were from University of Port Harcourt Teaching Hospital, Port Harcourt [UPTH] and Rivers State University Teaching Hospital, [RSUTH] Port Harcourt.

© 2020 Stella et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59396