Assessment of D-Dimer Levels in Southwestern Nigerian Pregnant Women

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors EOB and MAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DTA, GTO and OBW managed the analyses of the study. Authors EOB, DTA and OBW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluated D-dimer level in pregnant and non-pregnant women in Southwestern Nigeria in order to provide more information on the concentration and liable risks in this region.

Study Design: This is a cross sectional study where convenience sampling method was applied in sample collection.

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Place and Duration of Study: Blood samples were collected from pregnant women attending the antenatal clinics of Federal Teaching Hospital Ido-Ekiti (FETHI), Ekiti; Federal Medical Centre (FMC), Owo; and LAUTECH Teaching Hospital (LTH), Osogbo in Southwestern Nigeria.

Methodology: Exactly three hundred pregnant (300) and one hundred and fifty (150) apparently healthy non pregnant women were recruited for this study. The blood samples were analysed for haematocrit (HCT) and platelet count using Sysmex KX-21N (Japan); prothrombin time (PT) and activated partial thromboplastin time (APTT) by Diagen reagents (Diagnostic Ltd., UK); the international normalized ratio (INR) was calculated from the PT results; and D-dimer quantitative assay using Tina Quant Gen 2 on Cobas C111 (Roche). Data analysis was performed using IBM-SPSS version 25.0; mean and standard deviation was used to summarize continuous variables and descriptive and Inferential statistical tests were employed with level of statistical significance was determined at p<0.05.

Results: The mean D-dimer levels were significantly higher in the pregnant women (0.87 ± 1.00 ugFEU/ml) than in controls (0.31 ± 0.22 ugFEU/ml) with 42% of the pregnant population having elevated concentration while the mean PT, INR and HCT were significantly higher in controls than the subjects (p<0.05). Furthermore, the HCT, platelet, PT and INR were observed to be highest at first trimester; 36.04±5.09 (L/L), 182.72±35.11 (x109/L), 11.80±1.86 (seconds) and 0.35±0.15 respectively, decreasing across the second and the third trimester. On the other hand, the D-dimer and APTT increased exponentially from the first trimester; 0.42±0.18 (ugFEU/ml) and 30.80±3.30 (seconds), through the second and third trimesters respectively (p>0.05).

Conclusion: This study shows a significant increase in D-dimer in the pregnant subjects when compared with the control and an exponential increase in the third trimester, also a significant reduction in some other baseline coagulation profile hence depicting D-dimer as a notable significant marker of coagulation and fibrinolysis. This therefore emphasizes the hypercoagulable state of pregnancy and a need for adequate monitoring.

Keywords: D-dimer levels; pregnant women; blood samples; coagulation and fibrinolysis.

1. INTRODUCTION

D-dimer are double D fragments generated when activated factor XIII (FXIIIa) cross-linked fibrin is degenerated by plasmin which yield series of soluble fragments of varied molecular weights oligomers. The degradation process is a dynamic one yielding more fragments as the process proceeds. D-dimer fragments create neoepitopes which are revealed by specific antibodies used in the various analytic systems [1,2].

D-dimer being a tortuous marker of fibrinolytic activities also exhibits the potentials as marker of disorders of hemostasis and indicator of thrombotic disorders such as deep venous thrombosis and pulmonary embolism [2]. Generally, D-dimer has been extensively investigated routinely for the exclusion of venous thromboembolic disorders and evaluated for monitoring anticoagulation therapy in VTE, analysis and management of disseminated intravascular coagulation as well as other conditions in which the patient is highly predisposed to haemorrhage or thrombosis.

D-dimer assay elevation could result from collection of clinical scenarios such as pregnancy, age, cancer, surgery, activated protein C resistance, factor V Leiden and lack of clinical standardization [3].

The initial laboratory quantitative D-dimer assays were rooted in enzyme-linked immunosorbent assay (ELISA) technology which have undergone modification to coagulation and clinical chemistry auto-analyzers, with an endpoint dependent on latex enhanced, immunoturbidimetry, immunofluorescence and chemiluminescence methods [4,5]. Although these assays are exceedingly responsive and are economical to perform when analyzing large numbers of specimens, the combination of specimen transportation and analytical times often results in a prolonged turnaround time (TAT) which has initiated the rationale for the use of rapid D-dimer test kits in some countries presently.

Pregnancy condition being a hypercoagulable state is characterized by increase in some coagulation factors predisposing to thrombotic risk and consequential increased fibrinolysis. D-
The dimer has been employed in some parts of Nigeria to ascertain this claim using ELISA and latex suspension methods [5,6,7], however, there is need to assess the fibrinolytic and thrombotic risks of pregnant women in Southwestern Nigeria to provide more information on the hypercoagulable condition in order to enhance parturients antenatal monitoring and subsequent reduction in maternal mortality. In lieu of this, this study therefore determined the D-dimer concentration distribution among a substantial representative of the pregnant population across some states in this region.

2. MATERIALS AND METHODS

2.1 Study Population

In this cross sectional study, four hundred and fifty (450) subjects comprising of three hundred pregnant (300) women and one hundred and fifty (150) apparently healthy non-pregnant women were recruited from the antenatal clinics of three tertiary institutions across Southwestern Nigeria which are Federal teaching Hospital, Ido Ekiti (FETHI), Ekiti state; Federal Medical Centre (FMC), Owo, Ondo State and Ladoke Akintola University of Technology Teaching Hospital (LTH), Osogbo, Osun state. The participants across each centre were equally recruited comprising of one hundred pregnant subjects and fifty control subjects from each centre. The samples were collected in single measurements from the participants and the study was carried out for a period of nine months between November, 2018 and August, 2019. The population was age range 16 to 39 yrs, control participants with history of contraceptive use, smoking as well as pregnant women on anticoagulant therapy, history and symptoms of preeclampsia, gestational diabetes, eclampsia were excluded from this study. The non-pregnant control were female staff of the hospitals as well as female individuals who went to the clinics for employment medical checkup and volunteered as participants for the study. They were identified by their last menstrual period and negative result to hCG one step pregnancy test strips of Micropoint rapid diagnostic Test (Micropoint Diagnostics, Nantong, China, Lot hCG1907).

2.2 Sample Collection and Storage

Exactly 5 mls of whole blood was collected from each participant, 2 ml each was dispensed into ethylene diamine tetra acetic acid (EDTA) bottle for haematocrit (HCT) estimation and platelet count while the remaining 3 mls was dispensed in trisodium citrate anticoagulated vacutainer bottles and centrifuged at 1500 g for 15 minutes. The supernatant citrated plasma was aspirated into two eppendorf tubes and stored at -20°C for subsequent coagulation assays which are prothrombin time (PT), activated partial thromboplastin time (APTT) and D-dimer which were all analysed within 2 weeks of storage.

2.3 Platelet and Haematocrit Estimation

Sysmex KX-2 in Autoanalyser (Sysmex, 1999) was used to determine the HCT (L/L) and platelet count (cells/L) of the subjects in which a well-mixed EDTA anticoagulated blood was set at the sample probe and the start switch pressed. The sample was aspirated, analysis executed and the result printed.

2.4 Prothrombin Time

This was determined using the one-stage procedure with Diagen reagent (Diagnostic Ltd, UK, Lot number T84). A 200 µl of the calcium brain thromboplastin was dispensed into a clean test tube in a water bath at 37°C, 100 µl of the test plasma was added and the stopwatch was started simultaneously. The solution was well mixed and tilted until a clot was observed. The stopwatch was stopped immediately a clot was observed and the time recorded.

2.5 International Normalised Ratio (INR)

This was calculated as follows;

\[
\text{INR} = \frac{\text{PT Test}}{\text{PT Control}} \times \text{ISI}
\]

where ISI is 1.28 (Diagen Reagent, Diagnostic Ltd.)

2.6 Activated Partial Thromboplastin Time (APTT)

A 200 µl of the Diagen kaolin platelet substitute (Diagnostic Ltd., UK) was dispensed in a test tube in a water bath at 37°C, 100 µl of test sample was added, well mixed and incubated at 37°C for 2 minutes. Then 100 µl of preincubated 0.025 m CaCl was added and the stopwatch was started. The stopwatch was stopped immediately a clot was formed.

2.7 D-Dimer Assay

A quantitative particle-enhanced immuno turbidmetric assay was employed to determine
the D-dimer concentration. The D-dimer assay with Tina Quant Gen 2 reagent (cat no. 05077753-190) was performed on Cobas C111 (Roche) analyzer following the operator’s analyzer-specific assay instructions. Exactly 5 µl of plasma sample was diluted with 10 ul of water diluents, 90 µl of reagents R1 (TRIS/HCl buffer: 250 mmol/L) and SR (Latex particles coated with monoclonal anti-human D-Dimer antibodies (mouse)) were added to the dilution respectively. The absorbance of the turbid solution was read by the Cobas C111 (Roche Diagnostics Ltd.) and the concentration of the D-dimer was calculated and presented by the machine.

### 2.8 Statistical Analysis

Data analysis was done using IBM-SPSS version 25.0. Descriptive and Inferential statistical tests were employed. Data was summarized with frequency and proportions for categorical variables (age, gestational age) while mean and standard deviation was used to summarize continuous variables (PT/APTT, D-dimer, INR and HCT). Chi-square was used to test significance of association between categorical variables. The level of statistical significance was determined at p<0.05.

### 3. RESULTS

Table 1 represents the mean±SD of the parameters estimated for this study within the pregnant and control participants which displays the mean age (30 years) among subjects is lower than in controls (31 years) though statistically insignificant. However, the mean D-dimer were significantly higher in the pregnant women than in controls while the mean PT, INR, and HCT were significantly higher in controls than the subjects (p<0.05).

Furthermore, the HCT, platelet, PT and INR were observed to be highest at first trimester 36.04±5.09 (L/L), 182.72±35.11 (x10⁹/L), 11.80±1.86 (seconds) and 0.35±0.15 respectively, decreasing across the second and the third trimester with an insignificant statistical difference (p>0.05) (Table 2). On the other hand, the D-dimer and APTT increased exponentially from the first trimester (0.42±0.18 (ugFEU/ml) and 30.80±3.30 (seconds)) through the second (0.79±1.01 (ugFEU/ml) and 31.47±6.26 (seconds)) and third trimesters (1.13±1.02 (ugFEU/ml) and 32.62±6.06 (seconds) respectively (p>0.05).

#### Table 1. The mean ± standard deviation (SD) of parameters evaluated within the population

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameters (control range)</th>
<th>Subject (n = 300)</th>
<th>Control (n = 150)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age (16-39 years)</td>
<td>30.38 ± 4.35</td>
<td>31.48 ± 4.14</td>
<td>0.56</td>
</tr>
<tr>
<td>2</td>
<td>Platelet (222-312x10⁹/L)</td>
<td>171.38 ± 48.94</td>
<td>267.19 ± 42.07</td>
<td>0.00*</td>
</tr>
<tr>
<td>3</td>
<td>PT (11-14 seconds)</td>
<td>11.20 ± 1.89</td>
<td>11.94 ± 1.30</td>
<td>0.00*</td>
</tr>
<tr>
<td>4</td>
<td>INR (0.9-1.5)</td>
<td>0.96 ± 0.23</td>
<td>1.25 ± 0.46</td>
<td>0.00*</td>
</tr>
<tr>
<td>5</td>
<td>APTT (28-34 seconds)</td>
<td>32.23 ± 6.29</td>
<td>31.69 ± 1.93</td>
<td>0.31</td>
</tr>
<tr>
<td>6</td>
<td>D-dimer (0.1-0.5ugFEU/ml)</td>
<td>0.87 ± 1.00</td>
<td>0.31 ± 0.22</td>
<td>0.00*</td>
</tr>
<tr>
<td>7</td>
<td>HCT (33-43L/L)</td>
<td>33.67 ± 4.57</td>
<td>37.31 ± 5.71</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

Key: PT - Prothrombin time; APTT - Activated partial thromboplastin time; HCT - Haematocrit; INR - International Normalised Ratio

* Correlation is significant at the 0.05 level

#### Table 2. Comparison of the mean ± standard deviation (SD) of parameters estimated across the gestational periods of the pregnant women

<table>
<thead>
<tr>
<th>Parameters (control range)</th>
<th>Trimesters</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First (n=20)</td>
<td>Second (n = 207)</td>
</tr>
<tr>
<td>HCT (33-43L/L)</td>
<td>36.04±5.09</td>
<td>33.78±4.74</td>
</tr>
<tr>
<td>Platelet (222-312x10⁹/L)</td>
<td>182.72±35.11</td>
<td>176.81±46.54</td>
</tr>
<tr>
<td>PT (11-14 seconds)</td>
<td>11.80±1.86</td>
<td>11.03±1.59</td>
</tr>
<tr>
<td>APTT (29-33seconds)</td>
<td>30.80±3.30</td>
<td>31.47±6.26</td>
</tr>
<tr>
<td>D-dimer (0.10-0.50ugFEU/ml)</td>
<td>0.42±0.18</td>
<td>0.79±1.01</td>
</tr>
<tr>
<td>INR (0.9-1.4)</td>
<td>0.35±0.15</td>
<td>0.25±0.13</td>
</tr>
</tbody>
</table>

Key: PT - Prothrombin time; APTT - Activated partial thromboplastin time; HCT - Haematocrit; INR - International Normalised Ratio

Correlation is significant at the 0.05 level
In the pregnant women population, 20 (6.6%), 207 (69%) and 73 (24.09%) were in their first, second and third trimesters respectively, with variation in values and distribution of parameters assessed and observation that all the control subjects had normal values of D-dimer, PT and APTT as represented in Table 3. Exactly 26% and 13.4% of the pregnant subjects had increased D-dimer in their second trimester and third trimesters respectively while 43.6% had normal D-dimer and they are in their second trimester. Furthermore, 26% and 7.4% had reduced PT with 3.3% and 1.3% having elevated PT in their second and third trimesters respectively while 0.8%, 10.4% with 2.2% had decreased APPT and 0.7%, 2.6% and 1.0% having elevated APPT across the first, second and third trimester respectively (Table 3). These distributions display a significant difference across the trimesters at p equals 0.

Fig. 1 displays the distribution of the coagulation profile variation within the pregnant subjects and from the diagram it was observed that majority of the population had normal parameters across board whereas some had reduced or increased concentration of the profiles. It was observed that a high proportion (41.6%) of the subjects had increased D-dimer with the remaining 58.3% having normal concentration; over 30% and 20% had decreased PT and INR respectively and minute proportion having increased concentrations while those with decreased and increased APTT are 13 and 4.6% respectively.

**Table 3. Frequency distribution and comparison of D-dimer and other baseline coagulation profiles across the gestational stages in the pregnant and control populations**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference</th>
<th>Control</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>X²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (ugFEU/ml)</td>
<td>Normal</td>
<td>150(100%)</td>
<td>13(4.4%)</td>
<td>129(43%)</td>
<td>33(11%)</td>
<td>75.28</td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>0</td>
<td>7(2.2%)</td>
<td>78(26%)</td>
<td>40(13.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (seconds)</td>
<td>Decrease</td>
<td>150(100%)</td>
<td>4(1.4%)</td>
<td>78(26%)</td>
<td>22(7.4%)</td>
<td>58.88</td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0</td>
<td>16(5.2%)</td>
<td>119(39.2%)</td>
<td>47(15.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>0</td>
<td>0</td>
<td>10(3.3%)</td>
<td>4(1.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT (seconds)</td>
<td>Decrease</td>
<td>150(100%)</td>
<td>2(0.8%)</td>
<td>31(10.4%)</td>
<td>6(2.2%)</td>
<td>20.75</td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0</td>
<td>16(5.3%)</td>
<td>168(55.4%)</td>
<td>64(21.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>0</td>
<td>2(0.7%)</td>
<td>8(2.6%)</td>
<td>3(1.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Key:** PT - Prothrombin time; APTT - Activated partial thromboplastin time; HCT - Haematocrit; INR - International Normalised Ratio

*Correlation is significant at the 0.05 level
4. DISCUSSION

D-dimer is an important marker of thromboembolic disorder with a consequential intermittent increase in the disorder arising due to the occurrence of different triggering factors, including pregnancy, or genetic basis. Studies have evaluated the elevation of D-dimer in pregnancy [7-10] and illustrated the concentration of D-dimer amplified during gestation compared to non-gestation state with a gradual increase across the parturient period [11, 7].

From this study, it was revealed that the mean D-dimer is significantly higher in subjects than in controls with 42% of the pregnant population having increased D-dimer while the mean PT, INR, and HCT are significantly lower in subjects than in controls (p<0.05) (Table 1 and Fig. 1). This outcome may be emphasizing significant changes in haemostatic function during pregnancy as a result of increased production of coagulation factors with concurrent fibrinolysis and consequently reduced PT, INR with increased D-dimer level which is also established by several other studies assessing coagulation in pregnancy within Nigeria and worldwide [6-8, 12-15]. Similar studies established the fact that during normal parturieny, the haemostatic balance changes to yield hypercoagulability which in turn has been discovered to decrease bleeding complications in connection with delivery but uncontrolled hypercoagulation could initiate thrombotic complications in the condition [12-15].

Also, the platelet concentration in these researched subjects was observed to be significantly lower than the controls and this is suspected to be due to increased consumption of platelet in the parturients although the normal platelet value of each subject was not identified prior to the study and not monitored as the pregnancy progresses which may produce a more detailed information on the effect of pregnancy on platelet. However, some researchers made some observations that obstetrician and gynecologist considered reduced platelet count as a beginning of all coagulation disorders like preeclampsia and DIC. They stated that the more the decrease in platelet value, the more the severity of the case and these facts guide the physicians to use the platelet count as a baseline test for some coagulation disorders [12, 16].

Furthermore, this study observed an increasing drift in the D-dimer level from first to third trimester in the pregnant subjects along with a declining trend in similar manner in the platelet concentration which may be as a result of the fact that as the pregnancy progresses the physiological component of the pregnant body contributes to increased fibrinolysis because endothelial-derived plasminogen activator 1 (PAI-1) increases during the later stages of pregnancy just as placenta-derived PAI-2, which are detectable in the plasma from the first trimester, increases substantially throughout pregnancy [7, 17]; although this study involves a single measurement of parameters at a particular gestation age and this can be quite a substantial limitation in concluding that all the major changes in parameters assessed in each individual results from physiological composition of pregnancy. To corroborate these findings, several studies have also shown that the levels of D-dimer assays increase with gestational age and in complicated pregnancies as observed in preterm labor, abruptio placenta, and gestational hypertension [18, 19]. Also, the PT and INR decrease as the parturient period increases as observed across the trimesters and this may depict increased coagulation factors yielding reduced results (Table 2).

In addition, this study reveals that the highest number (68.3%) of the subject participants enrolled were in their second trimester followed by those in their first and third trimester respectively which may imply that the prenatal clinic attendance in this region is majorly attended and commenced in the second trimester. This observation was identified as a form of limitation in this study since some parameters were compared across gestational age. A similar finding has been observed by a previous demographic study by the National Demographic Health Survey where it was reported that antenatal clinics are being commenced by parturients in their second trimester. It was revealed that 18% of women had an antenatal clinic visit before four months of pregnancy and more than half made four months and above [20]. The survey also reported that one-third of births occur in public sector facilities and about one–third of births are assisted by a skilled provider such as birth attendant which is most common in Southwest Nigeria with the remaining births occurring at home [20]. So with this antenatal attendance from this study, the proportion of the pregnant subjects with elevated D-dimer progresses from first to thirds trimester.
just as the concentration increases implying that more pregnant subjects had elevated D-dimer as the gestational age progresses. However, majority of the subjects had standard D-dimer level in every gestational period. On the contrary, the number of the pregnant subjects with reduced PT and APTT increased with progressing gestational age while majority had normal values and few had elevated values.

In addition, it was observed from this study that 2 (0.7%) of the pregnant subjects had increased D-dimer reduced PT and APTT along with a consistent and severe lower limb pain and swelling which may be pointers to likely occurrence of deep vein thrombosis (DVT) if further investigations are done [21] since pregnancy is a risk factor to the occurrence, although it has been estimated that DVT occurs in about 0.5-1 in 1000 in pregnant women and it is five times more frequent than in non-pregnant women in the same age group [22]. Also, prolonged PT/APTT and increased D-dimer was observed in 4 (1.3%) of the subjects in their second trimester which are indicators of the likely onset of disseminated intravascular coagulation (DIC) a complication that could result into spontaneous haemorrhage if uncontrolled instantaneously and effectively. This is suspected because it has been reported earlier that regardless of the specific cause of DIC, the results are a malfunction of thrombin and prothrombin, which activate the fibrinolytic system; releasing clotting factors in the blood. DIC can interchange from hemorrhage to thrombosis and can exist as both, this situation further complicates diagnosis, management and treatment [23].

Maternal mortality is still ravaging the world with about 86% of such mortality occurring from the developing countries of Sub-Saharan Africa and Southern Asia [24] under bothersome and mostly preventable circumstances [25] and Nigeria’s maternal mortality is reported to be 545 per 100,000 births [20,26]. These mortalities results from direct and indirect causes [27] and the most common causes are complications from hemorrhage in about 27.1% of cases with more than 72.6 percent of the deaths classified as postpartum hemorrhage; while 14%, 10.7%, 7.9% and 12.8% results from hypertension, sepsis, abortion and embolism respectively [28]. Most of these causes can be prevented during antenatal visits if the coagulation profiles such as PT, APTT and D-dimer of these pregnant women are duly and comprehensively monitored based on the observations from this study. Such assessment will guide obstetricians at the pre- and post ‒ delivery stages of the subject hence contribute to a drastic reduction in maternal mortality especially in the low resource settings where high rate of preventable maternal deaths are been recorded.

5. CONCLUSION

This study shows a significant increase in D-dimer in the pregnant subjects when compared with the control and tremendous increase in the third trimester population, also a significant reduction in some other baseline coagulation profile were observed although there exist some associated limitations in the study since it is not a longitudinal study where the parameters are estimated from baseline pre-conception to postnatal period.

CONSENT AND ETHICAL APPROVAL

Ethical Approval was obtained from the ethical board/committee of FETHI, Ekiti; FMC, Owo; and LTH, Osogbo while informed consent was obtained from each participant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


