Assessment of Some Haemostatic Parameters in Women with Spontaneous Abortion Attending Antenatal Clinics in Yenagoa, Bayelsa State

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Authors’ contributions

This work was carried out in collaboration among all authors. Author EMM wrote the protocol, managed the literature searches and managed the analyses of the study, while author SUK-E wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at assessing some haemostatic parameters of women with spontaneous abortion attending antenatal clinics in Yenagoa, Bayelsa State.

Study design: This study is a crosssectional study.

Place and Duration of Study: This study was carried out in Federal Medical Centre (FMC) and Diete Koki Hospital, all in Yenagoa, Bayelsa State, Nigeria, between October 2018 and July 2019.

Methodology: A total of eighty-eight (88) subjects (48 women with spontaneous abortion and 40 women with normal pregnancy) were enrolled in this study. Ten (10) mls of blood sample was obtained from each participant. Platelet count and mean platelet volume were determined using the Mindray BC-5300 (Shenzhen Mindray Bio-Medical Electronics Co. Ltd China) Auto haematology analyzer. Prothrombin time was determined using Erba Actime invitro diagnostic reagent kits, activated partial thromboplastin time was determined using Erba Protime and Erba Calcium invitro diagnostic reagent kits, Fibrinogen was determined using Erba invitro reagents.

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Thrombin and antithrombin were determined using Erba Chrom kits while tissue plasminogen activator and tissue Plasminogen were determined using ELISA assay kits (Elabscience Biotech. Co. Ltd China). Data analysis was done using GraphPad Prism version 5.1 and p-values < 0.05 were considered statistically significant.

**Results:** Results showed a statistically significant decrease in aPTT (27.42 ± 7.827s versus 22.60 ± 7.038s) (P=0.003) among test and control groups irrespective of trimester, mean platelet volume (MPV) (10.76fl ± 1.89 versus 8.89 ± 1.00fl) (P = 0.004) among test and control groups in the first trimester. A significant decrease in activated partial thromboplastin time (28.44 ± 8.64s versus 21.82 ± 6.87s) (P=.0019) and antithrombin (32.57 ± 10.83% versus 16.34 ± 6.20%) (P =.001) among test and controls in the second trimester were observed. Also, a statistical decrease were observed in aPTT (32.00 ± 6.12s versus 20.36 ± 5.853s) (P=.0027) and (31.14 ± 9.083s versus 21.57 ± 9.343s) (P =.036) in the second trimester within the age ranges 18-23 years and 31-36 years. A significant negative correlations were observed in the test subjects for the first trimester between tissue in plasminogen and age mean platelet volume and platelet count and mean platelet volume and tissue plasminogen activator; second trimester test subjects between Platelet and age), antithrombin and platelet.

**Conclusion:** In conclusion, the findings suggest that the haemostatic parameters may contribute to complications in pregnancy.

**Keywords:** Haemostatic parameters; women; spontaneous abortion; antenatal clinics; Yenagoa, Bayelsa State.

## 1. INTRODUCTION

The word abortion is often used to mean only induced abortion. Abortion could be accidental, spontaneous or miscarriage of pregnancy as well as deliberate termination [1]. However, spontaneous abortion (SA) can be referred to as non-induced embryonic or foetal death or passage of product of conception before 20 weeks of pregnancy [1]. World Health Organization (WHO) defined spontaneous abortion (SA) as expulsion or extraction of a foetus or embryo weighing 500gm or less from its mother’s womb before 23 weeks of pregnancy [2]. In Nigeria, spontaneous abortion means the termination of pregnancy before 28 weeks from the last menstrual period [3].

In medical terms, spontaneous abortion also means miscarriage and starts with bleeding and pain may develop [4]. Spontaneous abortion (SA) is among the most common complication of pregnancy and occurring in about 10-15% of pregnancies [5]. The overall clinical recognized pregnancy that ends in miscarriage was estimated to be 12-15% [2], with the frequency increasing with rising maternal age and decreases with increasing gestational age [1]. In a study carried out in Nigeria in 2015, an estimated cases of spontaneous abortion was rated 4.2% between women age 19-49 [6-7]. Apparently, about (two thirds to three quarters) of clinical miscarriages in various studies occur during the first trimester [8]. Spontaneous abortion (SA) is termed early when it occurs before 12 weeks of gestation and late if it occurs between 12 weeks and 28 weeks of gestation [4]. There are various stages or types of spontaneous abortion namely; threatened, inevitable, incomplete and complete, missed abortion or fetal /embryonic dismiss [9].

The cause of spontaneous abortion (SA) is heterogeneous and not quite understood [10]. However, identifiable factors (chromosomal abnormalities, mutant genes), environmental toxins (drugs, lead and ionizing radiation), infectious agents (viruses, bacteria), uterine abnormalities (malformations, fibroids, cervical insufficiency and post-operative changes) and other maternal or paternal factors have been implicated [10]. Spontaneous abortion has been a great concern to the women folk; especially those that had experienced it once or twice. A lot of factors have been implicated. The establishment of haemostatic integrity of these women would help in the proper management of current spontaneous abortion as well as prevention of subsequent occurrences. Spontaneous abortion has been on the increase in Nigeria. A lot of factors are implicated, in Nigeria very few studies have been carried out on clinical findings related to spontaneous abortion. In South-South region, especially in Bayelsa State; there is dearth of knowledge on haemostatic profile of women with spontaneous abortion. It is believed that a better understanding of the haemostatic profile would
help in the proper diagnosis and management of spontaneous abortion in the area; hence, the aim of this study was to assess some haemostatic parameters of women with spontaneous abortion attending antenatal clinics in Yenagoa, Bayelsa State.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Federal Medical Centre (FMC) and Diite Koki Hospital, all in Yenagoa, Bayelsa State, Nigeria. Yenagoa is the capital city of Bayelsa State, Nigeria. It is located in coordinates 4°55’29”N 6°15’51”E [11], sitting on the equatorial belt with characteristics of high temperature, humidity, and heavy rainfall (3,000mm – 3,500mm) [12]. According to the 2016 National Population census, Yenagoa Local Government Area had a population of 352,285 and this figure was projected at 456,000 in 2015 (City Population, 2017). With an area of 706 km², this L.G.A. is bounded by Kolokuma/Opokuma L.G.A. on the North, Southern Ijaw LGA on the South, Sagbama Ijaw LGA on the North-west, and Ogbia LGA on the East (Iyarokpo, 2015). The official language is English, but Epie-Atissa Language is the major local language spoken in Yenagoa [11].

2.2 Determination of Sample Size

The sample size was determined using Leslie Kish’s formula:

\[ n = \frac{Z^2pq(1-p)}{d^2} \]

Where:
- \( n \) = is the sample size,
- \( Z \) = score statistics corresponding to the confidence interval (CI = 95%, \( Z = 1.96 \)),
- \( P \) = prevalence of spontaneous abortion obtained from a similar study (4.2%)
- \( q = (1- p) \) and,
- \( d = \) precision (5%).

The calculated sample size was 68 but because of the difficulty in getting the test subjects, a Convenience sample size of 48 was taken.

2.3 Study Population

A total of 88 participants of child bearing age between 18-45 years comprising of 48 women that had spontaneous abortion and 40 apparently healthy normal pregnant women as control all within 1-28 weeks gestation attending antenatal clinics in Yenagoa, Bayelsa State were recruited into this study. A structural questionnaire was employed to obtain the demographic data of the study participants.

2.4 Inclusion and Exclusion Criteria

All pregnant women between the age of 18-45 years who had spontaneous abortion served as the test group, while those with apparently healthy normal pregnancy were used as control.

Whilst, smokers, alcoholic, pregnant women with disease conditions such as cancer, diabetes and those on drugs capable of stimulating and causing the risk of abortion were excluded in this study.

2.5 Sample Collection

Six milliliters (6ml) of venous blood were collected through the ante-cubital vein of the arm from each participant using a standard venepuncture technique. Two milliliters (2.0ml) of the venous blood were dispensed into a glass bottle containing 0.5ml of 1.2mg/ml di-potassium ethylene diamine tetra-acetic acid (EDTA) and well mixed for platelet count, 2.5mls of venous blood was added into a glass tube containing 0.5ml of 32.0g/l trisodium citrate solution, properly mixed and processed for the determination of prothrombin time, activated partial thromboplastin time, fibrinogen and antithrombin, 1.5ml of the venous blood was dispensed into a non-anticoagulated plain bottle, for the determination of tissue plasminogen concentration and tissue plasminogen activator.

The blood samples in the sodium citrate containers were centrifuged at 2,500g for 10 minutes; the plasma was then separated into dry and clean plastic containers for the determination of prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and antithrombin, 1.5ml of the venous blood was dispensed into a non-anticoagulated plain bottle, for the determination of tissue plasminogen concentration and tissue plasminogen activator.

The blood samples in the sodium citrate containers were centrifuged at 2,500g for 10 minutes; the plasma was then separated into dry and clean plastic containers for the determination of prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and antithrombin (AT) within 6 hours of collection. The blood samples in di-potassium ethylene diamine tetra-acetic acid (EDTA) were analyzed within 2 hours of collection for platelet count. The blood samples in plain bottles without anticoagulant were allowed to clot for 2 hours to obtain serum, the serum was separated from the clotted blood and kept into a sterile clean container and stored overnight in a refrigerator at temperature between 2°C to 8°C, after which they were centrifuged at 2,500g for 10 minutes to
obtain a clear serum and used for the determination of tissue plasminogen and tissue plasminogen activator.

2.6 Experimental Analysis

2.6.1 Determination of platelets count and mean platelet volume

The platelets count and mean platelet volume were determined using Mindray BC-5300 (Shenzhen Mindray Bio-Medical Electronics Co. Ltd, China, 2017) Auto haematology Analyzer. The principle is based on electrical impedance method, which is measurement of changes in electrical resistance produced by a particle, which in this case is a blood cell, suspended in a conductive diluent as it passes through an aperture of known dimensions. An electrode is submerged in the liquid on both sides of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of electrical pulses generated signals the number of particles that passes through the aperture and the amplitude of each pulse is proportional to the volume of each particle. While the total number of each cell correlate with number of cells in the sample. The distribution of cell volumes is plotted on a histogram is by setting volume thresholds based on the typical size of each type of cell, the different cell population can be identified and counted.

2.6.2 Determination of prothrombin time

The Prothrombin Time was determined using Erba Actime invitro diagnostic reagent Kit (Erba Lachema s.r.o., Czech Republic, 2015). This is based on one-stage test of Owren, Erba (2015). The one stage prothrombin time (PT) measures the clotting time of plasma after adding a source of tissue factor (thromboplastin and calcium). The recalcification of plasma in the presence of tissue factor generates activated factor Xa (F Xa). Factor Xa in turn activates prothrombin to thrombin, which converts fibrinogen to an insoluble fibrin clot. The patient PT is compared to a normal standard.

2.6.3 Determination of activated partial thromboplastin time (aPTT)

The Activated Partial Thromboplastin Time was determined using Erba Actime and Erba Calcium Chloride solution 0.025M reagent (Erba Lachema s.r.o., Czech Republic, 2015).

By principle, the Activated Partial Thromboplastin Time (aPTT) test is performed by adding reagents containing a plasma activator and phospholipid to the test specimen. This mixture is incubated for 3 minutes at 37°C for optimum activation, calcium chloride is added and clot formation is timed. Clot detection can be done by mechanical, manual (tilt tube) or photo optical measurement.

2.6.4 Determination of fibrinogen

Fibrinogen was determined using Erba Thrombin reagent kit (Erba Lachema s.r.o., Czech Republic, 2015). This is based on Owren buffer method. The Owren buffer method determine the quantity of fibrinogen by measuring the clotting time of diluted plasma after the addition of thrombin >30 NIH units/ml. The clot time is proportional to the concentration of fibrinogen.

2.6.5 Determination of antithrombin III (AT-III)

Antithrombin was determined using Erba Chrom Antithrombin III reagent (Erba Lachema s.r.o., Czech Republic, 2015). The principle is based on chromogenic measurement. When factor Xa is added to a plasma dilution containing AT-III in the presence of excess heparin and calcium. After an initial incubation period, residual factor Xa is determined with a factor Xa-specific chromogenic substrate. The residual factor Xa activity is inversely proportional to the AT-III concentration.

2.6.6 Determination of tissue plasminogen concentration

Tissue Plasminogen was determined by ELISA methodology using Human Plasminogen ELISA kit, (Elabscience Biotech. Co. Ltd. China). The ELISA Kit makes use of Sandwich ELISA technique. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to human tissue plasminogen. Standards or samples are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human tissue plasminogen and Avidin-Horseradish peroxidase (HRP) conjugate are added successively to each microplate well and incubated. Free components are washed away. The substrate solution was added to each well. Only those wells that contain human tissue plasminogen biotinylated detection antibody and
Avidin-Horseradish peroxidase conjugate appeared blue in colour. The enzyme substitute reaction was terminated by the addition of stop solution which turns the colour to yellow. The optical density (OD) of the solution was spectrophotometrically measured at 450nm ±2nm wavelength. The value of the optical density was directly proportional to the concentration of the protein and the concentration of the protein in the solution calculated by comparing optical density of the solution to the standard by calculation.

### 2.6.7 Determination of tissue plasminogen activator concentration

Tissue Plasminogen Activator was determined by ELISA methodology using Human Tissue Plasminogen Activator ELISA kit, (Elabscience Biotech. Co. Ltd. China). The ELISA Kit makes use of Sandwich ELISA technique. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human Tissue Plasminogen Activator. Standards or samples are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human tissue plasminogen activator and Avidin-Horseradish peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human Tissue Plasminogen Activator biotinylated detection antibody and Avidin-Horseradish peroxidase conjugate will appear blue in colour. The enzyme substitute reaction will terminate by the addition of stop solution which will turn the colour to yellow. The optical density of the solution is spectrophotometrically measure at 450nm ±2nm wavelength. The value of the optical density is directly proportional to the concentration of the protein and the concentration of the protein in the solution calculated by comparing optical density of the solution to the standard by calculation.

### 2.7 Data Analysis

Data analysis was analyzed using the GraphPad Prism version 5.1. Results were expressed in Mean ± SD, correlations and p-values < 0.05 were considered statistically significant.

### 3. RESULTS AND DISCUSSION

Pregnancy is a hypercoagulable state and associated with remarkable changes in the entire haemostatic system, though most of the haemostatic changes in normal pregnancy return to pre-pregnancy state after birth. This study showed that the aPTT of test was significantly higher than that of control (P=.003) irrespective of trimester Table 1. The values (27.42±7.827seconds) (22.60±7.038 seconds) obtained for both the test and control groups were within the reference as described by [13]. Therefore, one would say that the values obtained in both the test and control are unlikely to produce any significant negative effect among women with spontaneous abortion in the area. This is not in agreement with the observation made by [14] were they predicted statistically significant difference with a mean value of (99.3 seconds ± 26.4 seconds) of 58 patients out of 261 that will have subsequent miscarriage. Also not in agreement with [15] were they observed a significantly lower aPTT level among the subject in their first and second trimesters when compared with control, (P =.011).

The findings from this study showed a significant difference in mean platelet volume (MPV) of the test (women with spontaneous abortion) when compared with control (P <.004) in their first trimester Table 2. The significant difference observed among the test and control groups has no clinical meaning, since the value fell within the upper normal limit. The significant increase of MPV in the test group could be due to activation of platelet that may arise from inflammatory reaction and bleeding in women with spontaneous abortion. This study is not in agreement with the study of [16-17].

The findings of this study also showed significant increase in aPTT of test when compared with the control in the second trimester (P=.0019). The significance increase on aPTT of test may be due to improper collection of sample and the method employed Table 3. Also, one would have said that, the significant increase may be due to endothelial damage that has resulted to bleeding but the values falls within the normal range. This is not in agreement with the finding of [14] were they predicted 58 patients with a mean value of 99.35 ± 26.4 seconds) that would have subsequent miscarriage. This finding also not in agreement with the study of [15] in their study of aPTT among pregnant women and non-pregnant women where they observed a statistically significant difference of aPTT level among the subject in the first and second trimester when compared with control (P =.011).
There was also a highly significant difference in antithrombin III among test subjects when compared with control subjects ($P < .001$). The increase level in AT-III found among the test group could be a reason for the test subjects experienced bleeding; but somehow the value falls within the normal limit. It could be due to low level of thrombin which could not interfere with the activity of AT-III to achieve a hypercoagulable state. There is also evidence of increased thrombin activity during pregnancy according to [17] but AT-III which is known to be the main inhibitors of thrombin and activated factor XII is not expected to show a compensatory rise during pregnancy. Inadequate fibrin formation resulting to hemorrhage can cause miscarriage in pregnancy too.

Table 1. Comparison of mean ± SD of haemostatic parameters of test and control irrespective of trimesters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>$\bar{x}$ ± SD</th>
<th>$p$ value</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLTC ($x10^6$/l)</td>
<td>Control</td>
<td>289.4 ± 171.7</td>
<td>0.0628</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>237.7 ± 74.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (s)</td>
<td>Control</td>
<td>12.98 ± 1.91</td>
<td>0.8124</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>13.11 ± 3.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT (s)</td>
<td>Control</td>
<td>22.60 ± 7.038</td>
<td>0.0034</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>27.42 ± 7.827</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>Control</td>
<td>281.6 ± 86.20</td>
<td>0.8946</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>284.1 ± 91.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>Control</td>
<td>33.19 ± 11.13</td>
<td>0.0596</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>37.34 ± 9.172</td>
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</tr>
<tr>
<td>Tissue Pla. (ng/ml)</td>
<td>Control</td>
<td>16.39 ± 6.455</td>
<td>0.8933</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>16.60 ± 7.921</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue Pla. Act. (ng/ml)</td>
<td>Control</td>
<td>6.044 ± 4.146</td>
<td>0.9887</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>6.055 ± 3.311</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>Control</td>
<td>11.18 ± 14.27</td>
<td>0.7160</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>10.42 ± 1.652</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: S=Significant, NS=Non-Significant, PLTC =Platelet Count, PT = Prothrombin Time, APTT= Activated Partial Thromboplastin Time, TP = Tissue Plasminogen, tPA = Tissue Plasminogen Activator, MPV = Mean Platelet Volume

Table 2. Comparison of mean ± SD haemostatic parameters of study subjects in the first trimester

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>$\bar{x}$ ± SD</th>
<th>$p$ value</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLTC ($x10^6$/l)</td>
<td>Control</td>
<td>291.4 ± 216.2</td>
<td>0.280</td>
<td>NS</td>
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<tr>
<td></td>
<td>Test</td>
<td>228.1 ± 69.8</td>
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<tr>
<td>PT (s)</td>
<td>Control</td>
<td>14.06 ± 4.78</td>
<td>0.424</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>12.92 ± 1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT (s)</td>
<td>Control</td>
<td>24.42 ± 7.39</td>
<td>0.699</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>25.38 ± 5.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>Control</td>
<td>292.2 ± 88.6</td>
<td>0.496</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>270.3 ± 78.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>Control</td>
<td>34.63 ± 12.16</td>
<td>0.627</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>36.47 ± 7.67</td>
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<tr>
<td>Tissue Pla. (ng/ml)</td>
<td>Control</td>
<td>16.7 ± 7.06</td>
<td>0.910</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>17.11 ± 10.81</td>
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</tr>
<tr>
<td>Tissue Pla. Act. (ng/ml)</td>
<td>Control</td>
<td>5.09 ± 3.46</td>
<td>0.389</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>6.30 ± 3.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>Control</td>
<td>8.89 ± 1.00</td>
<td>0.004</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>10.76 ± 1.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: S=Significant, NS=Non-Significant, PLTC =Platelet Count, PT = Prothrombin Time, APTT= Activated Partial Thromboplastin Time, TP = Tissue Plasminogen, tPA = Tissue Plasminogen Activator, MPV = Mean Platelet Volume
Table 3: Comparison of mean ± SD of haemostatic parameters of study subjects in the second trimester

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>( \bar{x} \pm SD )</th>
<th>p value</th>
<th>Remark</th>
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<tr>
<td>PLT (x10^6/l)</td>
<td>Control</td>
<td>288.6 ± 153.4</td>
<td>0.140</td>
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<td>Test</td>
<td>242.5 ± 77.85</td>
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<tr>
<td>PT(s)</td>
<td>Control</td>
<td>13.00 ± 2.21</td>
<td>0.333</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>12.57 ± 0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT(s)</td>
<td>Control</td>
<td>21.82 ± 6.87</td>
<td>0.0019</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>28.44 ± 8.64</td>
<td></td>
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<tr>
<td>Fibrinogen(mg/dl)</td>
<td>Control</td>
<td>277.0 ± 86.39</td>
<td>0.559</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>291.2 ± 98.32</td>
<td></td>
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</tr>
<tr>
<td>Antithrombin(%)</td>
<td>Control</td>
<td>16.34 ± 6.20</td>
<td>0.0001</td>
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<tr>
<td></td>
<td>Test</td>
<td>32.57 ± 10.83</td>
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<tr>
<td>Tissue Pla.(ng/ml)</td>
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<td>16.25 ± 6.32</td>
<td>0.957</td>
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<td></td>
<td>Test</td>
<td>16.34 ± 6.20</td>
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<tr>
<td>tPA(ng/ml)</td>
<td>Control</td>
<td>6.46 ± 4.40</td>
<td>0.597</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>5.94 ± 3.14</td>
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<tr>
<td>MPV(fl)</td>
<td>Control</td>
<td>12.16 ± 17.04</td>
<td>0.531</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>10.25 ± 1.52</td>
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</tr>
</tbody>
</table>

Key: S=Significant, NS=Non-Significant, PLTC=Platelet Count, PT=Prothrombin Time, APTT=Activated Partial Thromboplastin Time, tPA=tissue plasminogen activator, MPV=Mean Platelet Volume

Inadequacy in fibrin formation has been known to prevent implantation of the fertilized ovum in the uterus as stated by [18]. According to Hara and [19] during pregnancy, there is a little evidence that antithrombin-III decrease, therefore deficiency or increase are particularly at high risk of developing clot or bleeding during pregnancy or after delivery [20]. This is not in agreement with the observation made by [20] where he reported incidence of antithrombin deficiency ranging from 3% to 50%. Also this finding is not in agreement with that of [21] among Kosovo women with first trimester pregnancy loss.

A statistically significant negative correlation was observed between mean platelet volume (MPV) and platelets (PLT) count in the second trimester Table 5. The increased in MPV experienced by the test subjects may be due to increased platelet consumption during the remodeling of the foetus and uroplacental activities that has resulted to platelet swelling and pseudopodia formation. MPV is used as a platelet marker as it is easily measurable in vivo. This also means that the significant decrease in platelets count showed that MPV shows no apparent change in normal pregnancy. Therefore, the increase in MPV found among the test subjects is a normal physiological function and has no negative significant. This study is in agreement with the findings of [23], were MPV is found to be correlated with platelet count. Also agreed with the findings of [24] in their study of specific marker of coagulation, were they observed a significant increase in MPV \((P<.001)\) when compared with healthy control subjects.

The study showed a statistically significant negative correlation between mean platelet volume (MPV) and tissue plasminogen activator (tPA) of test subjects second trimester \((r =.540, P =.031)\). The increased in MPV may be due to increase in platelet aggregation which has altered platelet function that resulted to bleeding in women with spontaneous abortion. In normal pregnancy, a slight increase in platelet aggregation is observed is compensated by increased synthesis and consequently increase MPV.
Table 4. Correlation of haemostatic parameters with age of test subjects in the first trimester

<table>
<thead>
<tr>
<th>R values (p values)</th>
<th>PLT `count (x10^6/l)</th>
<th>Age(years)</th>
<th>PT(s)</th>
<th>APTT(s)</th>
<th>Fib(mg/dl)</th>
<th>Antithrombin(%</th>
<th>TP (ng/ml)</th>
<th>tPA(ng/ml)</th>
<th>MPV(fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PLT count(x10^6/l)</strong></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Age(years)</strong></td>
<td>0.004 (0.987)</td>
<td>0.279 (0.295)</td>
<td>0.033 (0.902)</td>
<td>0.394 (0.131)</td>
<td>-0.099 (0.713)</td>
<td>0.039 (0.033)</td>
<td>0.485 (0.057)</td>
<td>-0.557 (0.025)</td>
<td>0.292 (0.271)</td>
</tr>
<tr>
<td><strong>PT(s)</strong></td>
<td>-0.002(0.987)</td>
<td>-0.620 (0.819)</td>
<td>0.253 (0.343)</td>
<td>0.075 (0.781)</td>
<td>0.284 (0.285)</td>
<td>0.041 (0.879)</td>
<td>0.113 (0.674)</td>
<td>-0.534 (0.033)</td>
<td>0.479 (0.479)</td>
</tr>
<tr>
<td><strong>APTT(s)</strong></td>
<td>0.279 (0.295)</td>
<td>-0.062 (0.819)</td>
<td>0.253 (0.343)</td>
<td>0.075 (0.781)</td>
<td>0.284 (0.285)</td>
<td>0.041 (0.879)</td>
<td>0.113 (0.674)</td>
<td>-0.534 (0.033)</td>
<td>0.479 (0.479)</td>
</tr>
<tr>
<td><strong>Fib(mg/dl)</strong></td>
<td>0.394 (0.131)</td>
<td>0.075 (0.781)</td>
<td>0.253 (0.343)</td>
<td>0.075 (0.781)</td>
<td>0.284 (0.285)</td>
<td>0.041 (0.879)</td>
<td>0.113 (0.674)</td>
<td>-0.534 (0.033)</td>
<td>0.479 (0.479)</td>
</tr>
<tr>
<td><strong>Antithrombin(%</strong></td>
<td>-0.099 (0.713)</td>
<td>0.284 (0.285)</td>
<td>0.075 (0.781)</td>
<td>0.253 (0.343)</td>
<td>0.284 (0.285)</td>
<td>0.041 (0.879)</td>
<td>0.113 (0.674)</td>
<td>-0.534 (0.033)</td>
<td>0.479 (0.479)</td>
</tr>
<tr>
<td><strong>TP (ng/ml)</strong></td>
<td>-0.03 (0.910)</td>
<td>-0.062 (0.819)</td>
<td>0.253 (0.343)</td>
<td>0.075 (0.781)</td>
<td>0.284 (0.285)</td>
<td>0.041 (0.879)</td>
<td>0.113 (0.674)</td>
<td>-0.534 (0.033)</td>
<td>0.479 (0.479)</td>
</tr>
<tr>
<td><strong>tPA (ng/ml)</strong></td>
<td>0.485 (0.057)</td>
<td>0.075 (0.209)</td>
<td>0.253 (0.343)</td>
<td>0.075 (0.781)</td>
<td>0.284 (0.285)</td>
<td>0.041 (0.879)</td>
<td>0.113 (0.674)</td>
<td>-0.534 (0.033)</td>
<td>0.479 (0.479)</td>
</tr>
<tr>
<td><strong>MPV(fl)</strong></td>
<td>-0.557 (0.025)</td>
<td>0.292 (0.271)</td>
<td>0.158 (0.558)</td>
<td>0.120 (0.657)</td>
<td>0.120 (0.657)</td>
<td>0.158 (0.558)</td>
<td>0.120 (0.657)</td>
<td>-0.036 (0.892)</td>
<td>0.044 (0.869)</td>
</tr>
</tbody>
</table>

**KEY:** * = Correlation coefficient (r) for P < 0.05, PLT = Platelet Count, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, tPA = tissue plasminogen activator MPV = Mean Platelet Volume
### Table 5. Correlation of haemostatic parameters with age of test subjects in the second trimester

<table>
<thead>
<tr>
<th>R values (p values)</th>
<th>PLT Count (x10⁶/l)</th>
<th>Age (yrs)</th>
<th>PT (s)</th>
<th>APTT (s)</th>
<th>Fib (mg/dl)</th>
<th>Antithrombin (%)</th>
<th>TP (ng/ml)</th>
<th>tPA (ng/ml)</th>
<th>MPV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT Count (x10⁶/l)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.423 (0.016)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (s)</td>
<td>0.079 (0.677)</td>
<td>0.116 (0.540)</td>
<td>1</td>
<td>0.191 (0.311)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT (s)</td>
<td>0.098 (0.593)</td>
<td>0.122 (0.503)</td>
<td></td>
<td>0.148 (0.432)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fib (mg/dl)</td>
<td>-0.003 (0.987)</td>
<td>-0.079 (0.671)</td>
<td></td>
<td>-0.012 (0.947)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>-0.631 (0.000)</td>
<td>0.268 (0.144)</td>
<td></td>
<td>-0.030 (0.872)</td>
<td>-0.064 (0.737)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP (ng/ml)</td>
<td>-0.222 (0.221)</td>
<td>0.147 (0.419)</td>
<td></td>
<td>-0.065 (0.722)</td>
<td>0.241 (0.190)</td>
<td>0.414 (0.021)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>-0.171 (0.347)</td>
<td>0.122 (0.503)</td>
<td></td>
<td>-0.287 (0.111)</td>
<td>0.210 (0.255)</td>
<td>0.225 (0.222)</td>
<td>-0.031 (0.866)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>-0.270 (0.134)</td>
<td>0.078 (0.669)</td>
<td></td>
<td>-0.220 (0.226)</td>
<td>-0.111 (0.550)</td>
<td>0.115 (0.536)</td>
<td>0.030 (0.870)</td>
<td>-0.112 (0.540)</td>
<td>1</td>
</tr>
</tbody>
</table>

Key: * = Correlation coefficient (r) for P < 0.05, PLT = Platelet Count, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, tPA = tissue plasminogen activator MPV = Mean Platelet Volume

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Marcus et al.; IJR2H, 4(1): 53-66, 2021; Article no.IJR2H.66961

61
Table 6. Correlation of haemostatic parameters with age of control subjects in the first trimester

<table>
<thead>
<tr>
<th>r values (p values)</th>
<th>Age (yrs)</th>
<th>PLT Count (x10^6/l)</th>
<th>PT (s)</th>
<th>APTT (s)</th>
<th>Fibr (mg/dl)</th>
<th>Antithrombin (%)</th>
<th>TP (ng/ml)</th>
<th>tPA (ng/ml)</th>
<th>MPV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT</td>
<td>-0.1130.724</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (s)</td>
<td>0.300(0.343)</td>
<td>0.474(0.119)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT (s)</td>
<td>0.231(0.469)</td>
<td>0.604(0.037)</td>
<td>-0.269(0.397)</td>
<td>-0.284(0.370)</td>
<td></td>
<td>-0.084(0.795)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibr (mg/dl)</td>
<td>-0.082(0.799)</td>
<td>-0.269(0.397)</td>
<td>-0.486(0.109)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>0.110(0.732)</td>
<td>0.216(0.499)</td>
<td>0.467(0.125)</td>
<td>0.175(0.586)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP (ng/ml)</td>
<td>-0.001(0.997)</td>
<td>-0.121(0.706)</td>
<td>-0.175(0.586)</td>
<td></td>
<td></td>
<td></td>
<td>0.119(0.711)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>-0.121(0.707)</td>
<td>-0.161(0.615)</td>
<td>-0.144(0.655)</td>
<td>0.192(0.549)</td>
<td>0.277(0.383)</td>
<td>-0.030(0.026)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>0.101(0.753)</td>
<td>0.380(0.222)</td>
<td>0.380(0.222)</td>
<td>0.153(0.634)</td>
<td>0.4400.151</td>
<td>0.2730.389</td>
<td>-0.0320.919</td>
<td>0.2500.4</td>
<td>1</td>
</tr>
</tbody>
</table>

Key: * = Correlation coefficient (r) for P < 0.05, PLT = Platelet Count, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, tPA = tissue plasminogen activator MPV = Mean Platelet Volume
Table 7. Correlation of haemostatic parameters with age of control subjects in the second trimester

<table>
<thead>
<tr>
<th>r values (p values)</th>
<th>Age(yrs)</th>
<th>PLT Count (x10⁶/l)</th>
<th>PT(s)</th>
<th>APTT(s)</th>
<th>Fibrinogen (mg/dl)</th>
<th>Antithrombin (%)</th>
<th>TP (ng/ml)</th>
<th>tPA (ng/ml)</th>
<th>MPV(fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age(yrs)</strong></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT Count(x10⁶/l)</td>
<td>-0.05</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.765)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT(s)</td>
<td>-0.092</td>
<td>0.110(0.574)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.641)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT(s)</td>
<td>0.061</td>
<td>-0.075</td>
<td>0.043(0.824)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.754)</td>
<td>(0.704)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fib(mg/dl)</td>
<td>0.058</td>
<td>0.363</td>
<td>0.086</td>
<td>0.105(0.594)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.768)</td>
<td>(0.058)</td>
<td>(0.662)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin(%)</td>
<td>-0.066</td>
<td>0.190</td>
<td>-0.010</td>
<td>-0.016</td>
<td>0.148(0.452)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.736)</td>
<td>(0.333)</td>
<td>(0.958)</td>
<td>(0.934)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP(ng/ml)</td>
<td>0.131</td>
<td>-0.273</td>
<td>0.133</td>
<td>-0.022</td>
<td>0.106</td>
<td>0.032</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.506)</td>
<td>(0.159)</td>
<td>(0.499)</td>
<td>(0.910)</td>
<td>(0.589)</td>
<td>(0.868)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>-0.019</td>
<td>-0.073</td>
<td>-0.347</td>
<td>0.358</td>
<td>0.167</td>
<td>-0.140</td>
<td>-0.228</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.922)</td>
<td>(0.712)</td>
<td>(0.070)</td>
<td>(0.061)</td>
<td>(0.395)</td>
<td>(0.474)</td>
<td>(0.243)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPV(fl)</td>
<td>0.055</td>
<td>0.004</td>
<td>-0.185</td>
<td>-0.059</td>
<td>-0.099</td>
<td>0.116</td>
<td>-0.074</td>
<td>-0.071</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(0.779)</td>
<td>(0.981)</td>
<td>(0.345)</td>
<td>(0.765)</td>
<td>(0.615)</td>
<td>(0.555)</td>
<td>(0.708)</td>
<td>(0.719)</td>
<td></td>
</tr>
</tbody>
</table>

Key: * = Correlation coefficient (r) for P < 0.05, PLT = Platelet Count, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, tPA = tissue plasminogen activator MPV = Mean Platelet Volume
Increase in MPV of > 9.95fl has been indicated as a potential marker for predicting the severity of pre eclampsia in early pregnancy; though none of these women with spontaneous abortion were found to have pre-eclampsia. This study is not in agreement with the study of [25] where the MPV values in spontaneous abortion women showed statistically significant lower values (8.99 ±1.47fl) than in control group (9.66 ± 1.64fl) (P < .001). But agrees with the study of [24] where they observed increased in MPV in patient with confirmed platelet activation compared to healthy control subjects.

The study showed a statistically significant negative correlation between age and platelets (PLT) count of test subjects. (Women with spontaneous abortion) in the second trimester (r = -.423, P=.016), but not significant in the control group correlation Table 7. The age related alterations in platelets count observed among the test subjects may result from increased platelets aggregation which may lead to increase thrombotic event in aged women with spontaneous abortion.

This is in line with the study of [26] where they measure platelet translocation behavior on VWF (Von Willebrand factor) and found that platelet behavior of females were altered with age. This means that advanced maternal age may be a risk factor for thrombosis. The study is in agreement with the findings of [27], were they observed maternal age to be a risk factor for thrombosis during pregnancy.

A negative correlation was also observed between antithrombin –III (AT – III) and platelets (PLT) count among the test subjects in the second trimester (r = -.631, P=.001). The increase level of antithrombin –III experienced by the spontaneous abortion women could hinder the activities of thrombin and platelets thereby preventing a hypercoagulable state that has resulted to bleeding which was experienced by the spontaneous abortion women. Normal pregnancy is characterized by an increase in circulating number of platelets [28]. Also in pregnancy antithrombin is expected to be slightly reduced therefore increase in the AT-III level may prevent the action of thrombin thereby preventing a hypercoagulable state, This study is not in agreement with the study of [29] were they observed an inverse correlation between platelet adhesion and plasma antithrombin (r =.48 and r =.7) P< .05.

4. CONCLUSION

The findings of this study showed that the women with spontaneous abortion had a higher activated partial thromboplastin time (aPTT) than the control counterpart irrespective of trimester. From the investigation carried out on women diagnosed with spontaneous abortion, conclusion could be drawn that the haemostatic parameters may cause pregnancy complications; although the values fall within the normal ranges.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All authors declare that ‘written informed consent was obtained from the patient for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved (Approval no. FMCY/REC/ECC/2018/DEC/133) by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.
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Peer-review history:
The peer review history for this paper can be accessed here:
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