Blood Coagulation profile of pregnant women attending Ante-Natal Clinic at the University of Benin Teaching Hospital

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Abstract

Pregnancy induces extensive physiological changes in the haematological and haemostatic systems at the different trimesters. This is to accommodate the demands for foetal growth and development. To ascertain the changes in blood coagulation parameters in pregnancy, blood samples were randomly collected from 100 pregnant women in the three different trimesters who were attending the antenatal clinic at the University of Benin Teaching Hospital (UBTH), Benin City. The samples were analyzed for prothrombin time (PT), platelet concentration (PLT), activated partial thromboplastin time (APTT), haemoglobin concentration (HGB), white blood cell count (WBC) and packed cell volume or haematocrit (PCV or HCT). The values obtained were compared with the values in 50 non-pregnant women (control). The PT of 18.37 ± 0.3 seconds in pregnant women was significantly higher than 14.14 ± 0.2 seconds obtained in the control (p < 0.05). The PCV of 29.33 ± 0.3% obtained for pregnant women was significantly lower than 35.07 ± 0.2% in the control (p < 0.05) indicating a decrease in the PCV of pregnant women. Also, the APTT of 45.48 ± 1.1 seconds obtained from pregnant women was of no significance compared to 42.02 ± 0.3 seconds from the control (p > 0.05). Platelet concentration of 129.02 ± 3.5 x 10^9/L (pregnant women) was significantly lower than 175.10 ± 2.9 x 10^9/L (non-pregnant women). The results showed that the subjects had features of a hyper-coagulation state that might have been triggered by body immune reactions arising from low concentrations of most blood components and an increase in blood coagulation factors.

Introduction

Pregnancy is the fertilization and development of one or more offspring, known as embryo or foetus, in a woman's uterus. It occurs as a result of the fusion of the spermatozoon with a mature ovum during ovulation (Philip, 2012). It induces extensive physiological changes in the haematological and haemostatic system which in turn produces a vulnerable state for intravascular coagulation (Buseri et al., 2008). While these changes are aimed at minimizing intra-partum blood loss, they also increase the risk of some positive and negative blood conditions during pregnancy and the post-partum period (Hui and Lili, 2012).

Pregnancy is associated with changes in haemostasis which includes a decrease in platelet, an increase in the majority of clotting factors, a decrease in the quantity of natural anticoagulants and a reduction in fibrinolytic activity (Bremer, 2003; O’Riordan and Higgins, 2003). These changes result in a state of hypercoagulability, likely due to hormonal changes and increase the risk of thromboembolism. The risk of severe haemorrhage in pregnancy is well recognized, and uncontrolled bleeding occupies an important position in the etiology of maternal mortality and therefore, remains a major problem among several other causes of maternal mortality throughout the world (John, 2009). According to Hellgren (2003), haemorrhage accounted for 34.6% in the North Central Nigeria and 32.2% in Benin Republic.

The extent to which normal pregnancy affects coagulation parameters is not well documented in most localities. The objectives of this study were to assess the effect of normal pregnancy on some coagulation parameters, to determine the relationship between the gestation (trimester) period and the coagulation parameters.

Materials and methods

Sample collection

The study involved two groups of women: 100 pregnant women in 13th - 40th gestational weeks (GW) and a control group of 50 non-pregnant women. The subjects were randomly chosen from the general population of pregnant women attending the antenatal clinic at the University of Benin Teaching Hospital (UBTH) Benin City, while the control were also randomly selected from non-pregnant women among the general public in Benin City, Edo State.

Inclusion for Test (Pregnant women)

Healthy women age above 20 and below 35 years in the first, second and third trimesters, with no history of significant medical problems.

Inclusion for Control

Healthy women aged between 20 and 35 years, with no history of significant medical illness, and agreed to participate for the study voluntarily.

Analysis of Haematological and Coagulation Parameters

Haemoglobin Concentration (HGB)

The Cyanmethaemoglobin method (Dacie and Lewis, 2006) was used. A 0.02 ml of sample of well-mixed blood was placed in a test tube and 4 ml of Drakbin’s solution added. The blood sample and the Drakbin’s solution were mixed properly and left on the bench at room temperature for 10 minutes. The absorbance was read at a wavelength of 540 nm. The haemoglobin concentration (g/l) was calculated using the formula shown below:

\[
\text{HGB} = \frac{\text{Absorbance of test } \times \text{ Concentration of standard } \times 200}{\text{Absorbance of standard } \times 100}
\]

Packed Cell Volume (PCV) or Haematocrit (HCT)

The PCV was determined by following the method of Dacie and Lewis (2006). Anticoagulated blood in a glass capillary tube of specified length bore size and wall-thickness was centrifuged in a micro-haematocrit centrifuge at 12000 – 15000 g for 3-5 minutes, thus separating the blood into different components. Immediately after centrifugation, the PCV was read using the micro-haematocrit reader and the result was expressed as a percentage.

Platelets (PLT)

A volume of 0.38 ml ammonium oxalate was added to 0.02 ml of well mixed EDTA anticoagulated blood sample. The mixture was shaken manually for even mixing and left to stand on the
bench for 5 minutes (Cheesbrough, 2000). The counting chamber was used to count the PLT under the x10 and x40 objective lenses. The number of platelets counted was reported per litre of blood.

**White Blood Cells (WBC)**
A volume of 0.02 ml of well mixed EDTA anticoagulated blood sample was added to 0.38 ml of diluting fluid (Turks fluid), mixed properly, and left to stand on the bench for 5 minutes (Cheesbrough, 2000). The WBC were counted with a counting chamber under the x10 and x40 powers of the microscope and the number of WBC per litre of blood was recorded.

**Prothrombin Time (PT)**
The Quick’s one stage Prothrombin time method (Dacie and Lewis, 2006) was used. Using a 1ml pipette, 0.1 ml of test plasma was put into the pre-warmed test tubes in a water bath at 37°C and 0.2 ml of thromboplastin-calcium chloride mixture (PT reagent) was added. The test tube was tilted and checked for clot formation at intervals. Time was recorded in seconds

**Activated Partial Thromboplastin Time (APTT) or Prothrombin Thromboplastin Test with Kaolin (PTTK)**
Using a 1 ml pipette, 0.1 ml of the test citrated plasma was delivered into a 75 x 10 mm glass test tube placed in the water bath at 37°C (Cheesbrough, 2000). Then, 0.2 ml of the APTT reagent was added and left for 1-2 minutes in the water bath. Finally, 0.1 ml of calcium chloride was added and the reaction was timed using a stop-watch. The test tube was tilted and checked for clot formation at intervals.

**Statistical analysis**
The statistical evaluation was done by mean, standard deviation, paired t-test and ANOVA. Differences were considered significant with P-value less than 0.05 (p < 0.05). All statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) 20.0 statistical program. The single factor analysis of variance (ANOVA) was used to test the significant difference in the change in coagulation and haematological parameters for all the groups. Where significance difference was recorded, the Duncan Multiple Range Test was used to locate the source of the significant difference.

**Results**
Mean results and control are shown in Table 1. The comparison between some of the parameters in the control and pregnant women was statistically significant at p < 0.05. The PT increased significantly in pregnant women, with a mean value of 18.37 ± 2.7 seconds compared with the control who had a mean value of 14.14 ± 1.7 seconds. Pregnant women had a mean WBC count of 5.90 ± 0.12 x 10⁶/µl while the non-pregnant women had a mean value of 5.03 ± 0.08 x 10⁶/µl at; the difference was not significant. While the pregnant women had a mean PLT value of 175.10 ± 2.9 x 10⁶/µl, that of the control was 129.02 ± 3.5 x 10⁶/µl, showing a significant decrease. The APTT in pregnant women was not significantly altered compared to the control while HGB of pregnant women (9.6 ± 0.09 g/dl) was significantly different from that of control (12.02 ± 0.12).

![Figure 1: Haematological and coagulation parameter values in the first trimester](image1)

![Figure 2: Haematological and coagulation parameter values in the second trimester](image2)

### Table 1: Mean values of haematological and coagulation parameters in pregnant and non-pregnant women

<table>
<thead>
<tr>
<th>Haematological and Coagulation parameters</th>
<th>Non-pregnant women (Mean ± SE)</th>
<th>Pregnant women (Mean ± SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x 10⁶(µl)</td>
<td>5.03 ± 0.1</td>
<td>5.90 ± 0.1</td>
<td>p &lt; 0.05*</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>12.02 ± 0.1</td>
<td>9.69 ± 0.1</td>
<td>p &lt; 0.05*</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>35.07 ± 0.2</td>
<td>29.33 ± 0.3</td>
<td>p &lt; 0.05*</td>
</tr>
<tr>
<td>PLT x 10⁶(µl)</td>
<td>175.10 ± 2.9</td>
<td>129.02 ± 3.5</td>
<td>p &lt; 0.05*</td>
</tr>
<tr>
<td>PT (seconds)</td>
<td>14.14 ± 0.2</td>
<td>18.37 ± 0.3</td>
<td>p &lt; 0.05*</td>
</tr>
<tr>
<td>APTT (seconds)</td>
<td>42.02 ± 0.3</td>
<td>45.48 ± 1.1</td>
<td>p &lt; 0.05*</td>
</tr>
</tbody>
</table>

Note: Data presented are Mean ± standard Error of Mean; p > 0.05= No significant difference. * = significant

Abbreviations: HGB = Haemoglobin, HCT = Haematocrit, PLT = Platelet, WBC = White blood cell, PT = Prothrombin time, APTT = Activated Partial Thromboplastin Time.
in the haemoglobin concentration in all three trimesters is consistent with the report of Shen et al. (2010) showing a decrease in HGB which is as a result of haemodilution, in which there is an increase in plasma volume and no corresponding increase in blood cell components. This could be attributed to the additional progesterone and estrogen that are secreted by the placenta during pregnancy and this causes a release of renin from the kidneys. Renin stimulates the aldosterone-renin-angiotensin mechanism, leading to sodium retention and increased plasma volume. The increase in plasma volume is relatively greater than the increase in red cell mass, which results in a fall in maternal haemoglobin, hence the physiological anemia that occurs in pregnancy (Allen, 2007). In addition, the progressive decline in HGB concentration observed from the first to third trimesters may be due to an increased demand for iron as pregnancy progresses. More iron is required to meet the expansion of maternal haemoglobin mass and the needs of fetal growth (James et al., 2005).

Changes in coagulation system could be due to increased synthesis or increased activation by coagulation factors. These changes serve to protect the mother from the hazard of bleeding imposed by placenta and delivery, but they also carry the risk of an exaggerated response, localized or generalized (Bijoy et al., 1999). It has been observed that pregnant state results in significant increase in some coagulation parameters showing that pregnant state is a risk for hypercoagulability and should be managed and monitored so as to reduce maternal and neonatal morbidities. It is strongly recommended that coagulation profile be added as a periodical test, even if not as a routine test, for pregnant women during their antenatal period.

References


