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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 ± 1.1seconds 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 ± 2.9×10^9/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.

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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 spermatozoa 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 (Bremme, 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 aetiology 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 Drabkin's solution added. The blood sample and the Drabkin'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:

$$HGB = \frac{\text{Absorbance of test x Concentration of standard x 200}}{\text{Absorbance of standard x 100}}$$

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

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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 10^3/µl while the non-pregnant women had a mean value of 5.03 ± 0.08 x 10^3/µl at; the difference was not significant. While the pregnant women had a mean PLT value of 175.10 ± 20.6 x 10^6/µl, that of the control was 129.02 ± 34.9 x 10^6/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).

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

Haematological and Coagulation parameters	Non-pregnant women (Mean ± SE)	Pregnant women (Mean ± SE)	p-value
WBC x 10^3(µl)	5.03 ± 0.1	5.90 ± 0.1	$p < 0.05$*
HGB (g/dl)	12.02 ± 0.1	9.69 ± 0.1	$p < 0.05$*
HCT (%)	35.07 ± 0.2	29.33 ± 0.3	$p < 0.05$*
PLT x 10^3(µl)	175.10 ± 2.9	129.02 ± 3.5	$p < 0.05$*
PT (seconds)	14.14 ± 0.2	18.37 ± 0.3	$p < 0.05$*
APTT (seconds)	42.02 ± 0.3	45.48 ± 1.1	$p < 0.05$*

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.

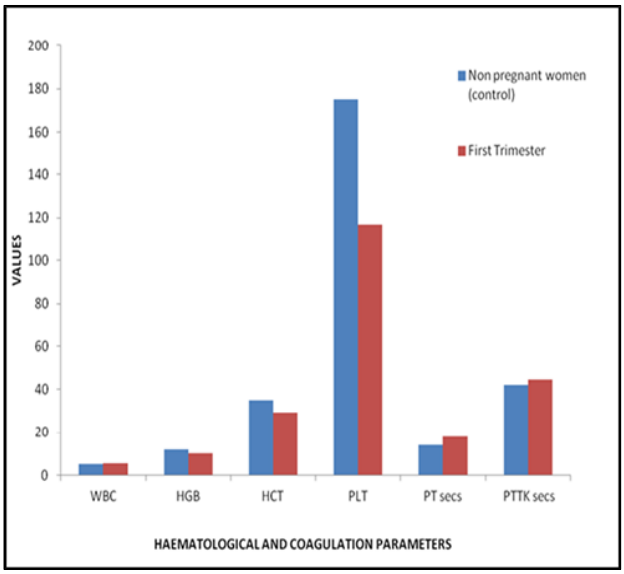

Figure 1: Haematological and coagulation parameter values in the first trimester

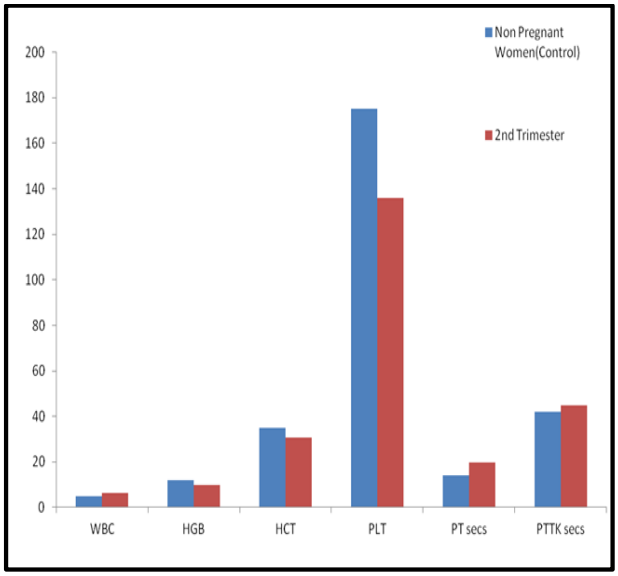

Figure 2: Haematological and coagulation parameter values in the second trimester

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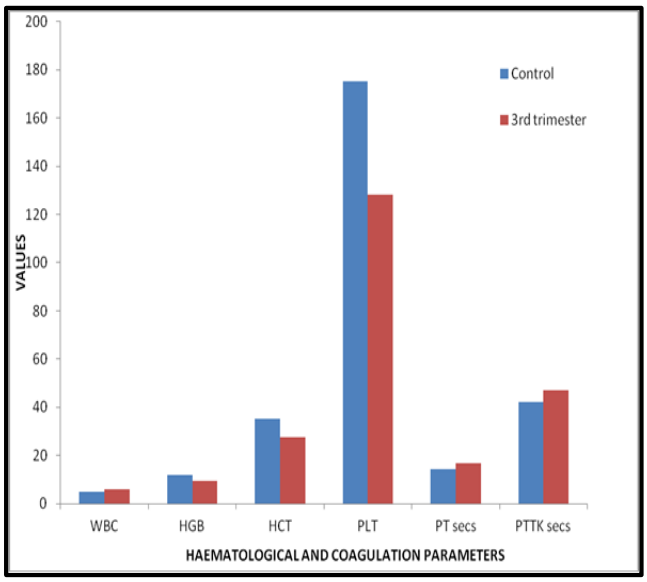

Figure 3: Haematological and coagulation parameter values in the third trimester

Duncan's Multiple Range Test showed that the third trimester was statistically different from the other trimesters in most of the parameters. Results showed a gradual reduction in PLT count as pregnancy advanced, with a significant decrease in the first trimester (mean PLT count of 116.96 ± 6.5 x 106/µl) which was lower compared to the second and third trimesters having a mean PLT value of 136.11 ± 5.0 x 106/µl and 128.23 ± 7.0 x 106/µl, respectively. The comparison between control and the different individual trimesters using the ANOVA test showed statistical significance in WBC, PLT counts, HCT, HGB and PT (Figures 1 - 3). When values for the first and second, first and third, second and third trimesters were compared with the control, there was no significant difference in most parameters. HGB, HCT and PT were significant for second and third trimesters when compared.

Discussion

This study identified a significant increase in Prothrombin time (PT) in pregnant women when compared with non-pregnant women. This is in agreement with the study carried out by Hellgren (2003), who reported increased endogenous thrombin generation and increased PT, and the work of Cerneca *et al.* (1997) that showed that the parameters having the greatest variation during pregnancy were PT and PLT. However, one study by Pal *et al.* (2010) showed that PT remains unchanged in pregnancy, which is contrary to our findings. The increased PT observed in this current report may be because of change in haemostatic balance in the direction of hypercoagulability in which there is increased concentration of all clotting factors except XI, XIII. (Hellgren, 2003). The total WBC in the pregnant and control are not in agreement with the study carried out by Onwukeme and Uguru (1999) who reported an increase in WBC from the first to third trimesters.

As pregnancy advanced, there was gradual reduction in PLT count. These findings corroborate those of a similar study undertaken by Akingbola *et al. (*2006) which reported exactly the same pattern. Due to hemodilution secondary to expansion of plasma volume, the PLT count in pregnancies may decrease by approximately 10%, with most of this decrease occurring during the first trimester (Boehlen *et al.,* 2000). The comparison between HCT in non- pregnant and pregnant women was shown to be statistically significant at $P < 0.05$. The result of this study showed significant reduction in HCT in pregnancy in all three trimesters. This is in line with a previous study carried out by Surabhi *et al.* (2012). The reduction in HCT occurs as a result of increased plasma volume associated with normal pregnancy, causing dilution of the whole blood without resultant effect of increase on cellular components of blood especially the red cells. The decrease 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 placentation 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

Allen, L. H. (2007). Anemia and iron deficiency: Effects on pregnancy outcome. American Journal of Clinical Nutrition 7 (5): 1280–1284.

Akinbami, A. A., Ajibola, S. O., Rabiu, K. A., Adewunmi, A. A., Dosunmu, A. O., Adediran, A., Osunkalu, V. O., Osikomaiya, B. I. and Ismail, K. A. (2013). Hematological profile of normal pregnant women in Lagos, Nigeria. International Journal of Women' Health 3 (5): 227-232.

Akingbola, T. S., Adewole, I. F., Adesina, O. A., Afolabi, K. A., Fehintola, F. A., Bamgboye, E. A., Aken'ova, Y. A., Shokunbi, W. A., Anwo, J. A. and Nwegbu, M. M. (2006).Haematological profile of healthy pregnant women in Ibadan, south-western Nigeria. Nigerian Journal of Obstetrics and Gynaecology 26 (8): 763–769.

Bijoy, S. S., Sisir, K. C., Hanes, G. T. and Partha, S. S. (1999). Obstetrics for Post graduates & Practitioners. Aspects of Fetal and Maternal Medicine.1st Edition. Churchill Livingstone. Philadelphia. Pp. 4-10.

Boehlen, F., Hohlfeld, P., Extermann, P., Perneger, T.V. and Moerloose, P. (2000). Platelet count at term pregnancy: a reappraisal of the threshold. Obstetrics and Gynecology 95: 29–33.

Bremme, K. A. (2003). Haemostatic changes in pregnancy. Best Practice and Research in Clinical Obstetrics and Haematology 16: 153-168.

Buseri, F. I., Jeremiah, Z. A. and Kalio, F. G. (2008). Influence of Pregnancy and Gestation Period on some Coagulation Parameters among Nigerian Antenatal Women. Research Journal of Medical Science 2 (6): 275-281.

Cerneca, F., Ricci, G. and Simeone, R. (1997).Coagulation and fibrinolysis changes in normal pregnancy. European Journal of Obstetrics, Gynecology and Reproductive Biology 73 (1):31-36.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries, Part 2.Low Price Edition. Cambridge University Press. Cambridge. Pp. 310-323.

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Dacie, J. W. and Lewis, S. M. (2006). Practical Haematology, 10th ed., Churchill Livingstone. Philadelphia. Pp. 13-18.

Hellgren M. (2003). Haemostasis during normal pregnancy and puerperium. Seminar on Thrombosis and Haemostasis 29 (2): 125-130.

Hui, C. and Lili, M. (2012). Changes in coagulation and hemodynamics during pregnancy: a prospective longitudinal study of 58 cases. Archives of Gynecology and Obstetrics 285 (5): 1231-1236.

James, A. H., Bushnell, C. D., Jamison, M. G. and Myers, E. R. (2005).Incidence and risk factors for stroke in pregnancy and the puerperium. Obstetrics and Gynecology 106: 509–516.

John, B. (2009). Haematological problems and Coagulation disorders in Pregnancy. Journal of Clinical Pathology 10: 35-41.

O'Riordan, M. N. and Higgins, J. R. (2003). Haemostasis in normal and abnormal pregnancy. Best Practice and Research in Clinical Obstetrics and Gynaecology 17: 385–396.

Onwukeme, K. E., Uguru, V. E. (1999). Haematological values in pregnancy in Jos. West African Journal of Medicine 9 (2): 70–75.

Pal, B., Szecsi, M., Jorgensen, A. K., Malene, R. A., Nina, P. C. and Steen, S. (2010). Haemostatic reference intervals in pregnancy. Thrombosis and Haemostasis 103 (4): 718-727.

Philip J.D. (2012). Clinical Gynecologic Oncology. Philadelphia: Lippincott Williams & Wilkins. 708 pp.

Shen, C., Jiang, Y. M., Shi, H. (2010). A prospective, sequential and longitudinal study of haematological profile during normal pregnancy in Chinese women. Journal of Obstetrics and Gynaecology 30 (4): 357–361.

Surabhi, C., Anil, K. T., Sanjay, M., Mohammad, A., and Arvind, K. V. (2012). Physiological Changes in Hematological Parameters during Pregnancy. Indian Journal of Hematology and Blood Transfusion 28 (3): 144–146.