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Influence of Pregnancy and Gestation Period on Some Coagulation Parameters among Nigerian Antenatal Women

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Abstract: There are two major objectives in this study. The first is to assess the effect of normal pregnancy on some coagulation parameters. The second is to determine the relationship between gestation (trimester) period and the coagulation parameters. Five parameters, platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (FIBR) and factor VII (FVIII) were assessed in 126 apparently health, pregnant women and 58 non-pregnant controls, using standard procedures. Pregnancy was found to exert significant increases in all the parameters; PLT ($p = 0.05$), PT ($p = 0.05$), aPTT ($p = 0.009$), Fibrinogen ($p<0.01$) and FVIII ($p = 0.03$). Prothrombin time correlated positively and significant with aPTT and FVIII ($p<0.01$ and $p<0.05$). A more significant and positive correlation existed between aPTT and FVIII ($p<0.001$). Regression line of best fit was determined between the five parameters and gestation period (trimester) for the purpose of establishing the relationship between them using multiple regression models. The gestation period (trimester) was found to relate polynomially with platelet count ($y = 0.0015x^3 - 0.1896x^2 + 8.5114x + 54.199$, $R^2 = 0.9729$) and exponentially with the other parameters (PT) $y = 9.5166e^{0.017x}$, $R^2 = 0.9823$, aPTT $y = 18.461e^{0.0239x}$, $R^2 = 0.9793$, FIBR $y = 76.999e^{0.0141x}$, $R^2 = 0.8704$, FVIII $y = 28.505e^{0.0221x}$, $R^2 = 0.9396$. We concluded that normal pregnancy state results in the significant increase in most coagulation parameters. This could lead to hypercoagulable state in some women if not properly checked. Increased maternal age (>40 years) could be a risk factor for hypercoagulability in pregnancy.

Key words: Pregnancy, prothrombin time, activated partial thromboplastin time, fibrinogen, platelet, factor VIII

INTRODUCTION

Normal pregnancy is often referred to as a hypercoagulable state in which changes in the haemostatic system has been reported (Holmes and Wallace, 2005; Eichinger *et al*., 1999). These changes are considered to be in preparation for the haemestatic challenge of delivery. Pregnancy is a risk factor for venous thrombosis and the incidence of venous thromboembolism during normal pregnancy is 6-fold higher than in the general female population of childbearing age (Lindqvist *et al*., 1999).

Virchow's triad postulates the principal factors underlying venous thrombosis: venous stasis, vascular damage and hypercoagulability all of, which occur during pregnancy (Pabinger *et al*., 2002). Venous stasis of the lower limbs occurs by the end of the first trimesters (Holmes and Wallace, 2005; Pabinger *et al*., 2002; Macklon and Greer, 1996).

Haemostasis in normal pregnancy involves a complex network of interactions with positive and negative feedback loops, integrating blood vessels; platelets, coagulation factors, coagulation inhibitors and fibrinolysis and has evolved to maintain the integrity of the vasculature. Normal pregnancy is associated with substantial changes in the tissue factor pathway and in the wider haemostatic system. Traditionally, it is proposed that these changes are in preparation for the haemostatic challenge of delivery, with the haemostatic system returning to that of the non-pregnant state of approximately 4 weeks of post delivery (Greer, 1994).

Among several other causes of maternal mortality, haemorrhage has been reported to be the major cause in the West Africa sub regions (Ujah *et al*., 2005; Jacques *et al*., 2006). In the two separate studies in the West African subregion, haemorrhage accounted for 34.6% in North Central Nigeria (Ujah *et al*., 2005) and 32.2% in Benin Republic (Jacques *et al*., 2006).

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To what extent normal pregnancy affects coagulation parameters is not well known in our locality. A study like this is therefore necessary to assess the influence of normal uncomplicated pregnancy on coagulation parameters. It is also necessary to know, the extent of relationship between the gestation period (trimesters) and the coagulation parameters, hence this study. It is hoped that the findings in this study will provide a base for future research and also assist professionals in the management of normal uncomplicated pregnancy.

MATERIALS AND METHODS

Subjects: The study population included 126 apparently healthy pregnant women, who visited the antenatal clinic of the Braithwait Memorial Specialist Hospital, Port Harcourt between October and December 2006. Fifty eight apparently healthy age-matched non-pregnant women served as controls for the study. Both subjects and controls were randomly chosen from the general population of pregnant women. All subjects gave informed consent to participate in the study, which was approved by the Ethical Committee of the Braithwait Memorial Specialist Hospital, Port Harcourt.

Collection of blood samples: Whole blood (4.5 mL) was collected by venepuncture into 0.5 mL of 3.2% trisodium citrate in a plastic tube and the plasma obtained by centrifugation at 2500 g for 15 min. The plasma was stored in stoppered tubes and used within 4 h. of collection.

Materials: Prothrombin time and aPTT tests were done using reagents bought from DIAGEN Diagnostics Reagents Limited, Thame, OXON, UK. Fibrinogen determination was carried out using Fibrinogen reagent Kit (Ref 5138005) purchased directly from TECHNOCLONE GmbH, Vienna, Austria. Factor VIII assay was done using Factor VIII deficient plasma obtained from TECHNOCLONE, GmbH, Vienna Austria. Platelet count was carried out manually using Haemocytometry.

Calibrations: Before the test procedures commenced, calibration curves for fibrinogen estimation and factor VIII assay were done. For fibrinogen calibration, the coagulation reference was reconstituted with 1ml distilled water. A geometric dilution of 1:5, 1:10, 1:20 and 1:40 was made using imidazole buffer. The coagulation times of these dilutions were determined. The fibrinogen concentratiosn in mg/100 mL was calculated by multiplying the given value by 10 and dividing by the appropriate dilution factor. A calibration curve was plotted on double log paper with fibrinogen concentration (mg/100 mL) on x-axis and coagulation time (in sec) on y-axis. The values (coagulation time in seconds) of the 1 in 10 diluted plasma were read off the calibration curve directly.

For factor VIII calibration curve, the coagulation reference was reconstituted as indicated in the manufacturers product insert. A predilution of 1 in 5 is attained by using an imidazole buffer in the ratio of 1:5. A geometric series of dilutions (1:1-1:128) of the 1:5 predilution was made. The 1:1 corresponds to the predilution of 1:5. The coagulation times of the geometric series of dilutions was plotted on semilog paper. Activity in percent on x-axis and coagulation times in seconds on y-axis.

Methods

Platelet counts: Platelet count was done using the ICSH approved procedures (ICSH, 1986). Well mixed whole blood was diluted with 1% ammonium oxalate in the ratio of 1 in 20 (50 μL of blood to 950 μL of anticoagulant). The haemocytometer was then filled with an aliquot of this mixture and allowed to settle for 1 min prior to performing the count. Platelets were counted in 10 small central squares and the number calculated using the first principle.

Cells counted × dilution factor × chamber depth/ area of chamber counted. Results were expressed as the number of platelets × 109/L (SI units).

Prothrombin Time (PT): The one stage procedure was employed in PT determination. 0.2 mL of thromboplastin-calccium reagent was placed in the clotting tube in a waterbath at 37°C and left for 2 or 3 min. to reach 37°C. 0.1 mL of plasma was then added and a stop watch started. The tube was then gently titled at 2-3 sec intervals and the time for the formation of a clot recorded.

Activated Partial Thromboplastin Time (aPTT): About 0.2 mL of Kaolin platelet substitute was placed in a clotting tube and 0.1 mL of plasma added and the tube gently tilted at intervals for exactly two minutes. 0.1 mL of 0.025 mL calcium chloride was then added and a stopwatch started. The tube was tilted 3-5 sec. interval and the clotting time recorded. The test was carried out, in duplicate for both normal control and the subjects and the mean value obtained.

Fibrinogen determination: The determination of fibrinogen level was done using the modified clauss method. In this method, citrated plasma is diluted, mixed with an excess of thrombin and the coagulation time determined. Patient and control plasmas were diluted 1 in

10 with imidazole buffer (1 volume of plasma to 9 volume of buffer) 0.2 mL of the citrated plasma was pipetted into the test tube and incubated for 1 min. at 37°C). About 2 mL of the fibrinogen reagent was added to the citrated plasma and the point of coagulation was determined using the stopwatch. Results were extrapolated from the calibration curve.

Factor VIII assay: Factor VIII deficient plasma was used in the determination of coagulation factor VIII by one stage method used in the activated partial thromboplastin time (aPTT). Factor VIII deficient plasma is a lyophilized stabilized human haemophilia A plasma with factor VIII content <1%. Before the test was carried out, the subjects plasma was diluted 1:5 using the imidazole buffer. About 0.1 mL of factor VIII deficient plasma was added to 0.1 mL of diluted plasma and then 0.1 mL of PTT reagent added. The mixture was allowed to incubate for 3 min at 37°C. After the incubation, 0.1 mL of 25 Mmol calcium chloride was added and the point of coagulation determined immediately using the stopwatch. Results were read using the calibration curve.

RESULTS

The plasma levels of five coagulation parameters in 126 pregnant women and 58 non-pregnant controls were assessed. Table 1 shows the summary of the mean values of the five parameters tested. The platelet counts of 221.6±97.0×10^9/L among pregnant women was found to be significantly higher than 179.8±107.9×10^9/L among non-pregnant controls (F = 3.435, p = 0.05). Prothrombin time, 25.3±13.3 sec in pregnancy was equally higher than 19.9±10.4 sec in control subjects. Similar, increased values were observed in aPTT and Factor VIII concentrations (aPTT, 77.3±53.3 versus 48.8±28.8 sec Factor VIII assay, 109.2±98.4 versus 68.4±31.1%) ($p<0.05$). The fibrinogen concentration among pregnant women, 174.0±101.5 was also statistically different from 157.0±59.2 mg 100 mL^{-1} among non-pregnant controls (F = 6.723, p = 0.01).

Table 2 shows the mean square table from ANOVA for sources of variation. Sample (for both subjects and controls) was found to contribute to variations in prothrombin time, aPTT and factor VIII, while platelet count and fibrinogen level were not affected. The age of the subjects contributed to the variations only in factor VIII concentration.

The effect of age on coagulation parameter in pregnancy is shown in Table 3. The Duncan test of significance did not reveal any statistical significant effect on the parameters tested ($p>0.05$). Similarly, there was no significant effect of trimesters on the coagulation parameters in pregnancy as shown in Table 4 ($p>0.05$), Pearson correlation of the five variables is as shown in Table 5. Prothrombin time correlated positively and significantly with aPTT and factor VIII ($p<0.01$ and $p<0.05$). A more highly statistically significant and positive correlation was observed between aPTT and factor VIII concentration ($p<0.001$). Multiple regression analysis was performed to establish the relationship existing between the five parameters and the trimester.

Table 1: Mean values of the five coagulation parameters tested among pregnant women and control subjects

Parameters	Pregnant women n = 126 x ± SD	Controls n = 58 x ± SD	F-value (Between groups)	Sig.
Platelet count (× 10^9/L)	221.6±697.0	179.8±107.9	3.435	0.05*
Prothrombin time (sec)	25.3±13.30	19.9±10.40	3.684	0.05*
aPTT (sec)	77.3±53.30	48.8±28.90	7.205	0.009**
Fibrinogen (mg 100 mL^{-1})	174.0±101.5	157.0±59.20	6.723	0.01**
Factor VIII assay (%)	109.2±98.40	68.4±31.10	4.738	0.03*

* = $p<0.05$, ** = $p<0.01$

Table 2: Mean square table from ANOVA for sources of variation

Source of variation	Dependent variables	DF	Mean square	F-value	Sig.
Sample	Platelet count	1	8279.900	0.789	0.377^{ns}
(Subjects and	PT	1	777.400	4.660	0.03*
controls)	aPTT	1	19793.800	9.760	0.002**
	Fibrinogen	1	9906.900	1.183	0.280^{ns}
	Factor FIII	1	100021.200	26.616	0.000***
Age	Platelet count	5	10894.500	1.039	0.301^{ns}
	PT	5	59.164	0.355	0.878^{ns}
	aPTT	5	3942.500	1.944	0.096^{ns}
	Fibrinogen	5	5450.800	0.651	0.662^{ns}
	Factor VIII	5	20938.100	5.572	0.000***

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, PT = Prothrombin time, aPTT = Activated partial thromboplastin time

The relationship between platelet count and trimester was polynomial to the third order (R^2 = 0.9728), while prothrombin time, aPTT, fibrinogen and factor VIII concentrations exhibited exponential relationships with the trimesters in pregnancy. The equations for the line of best fit are as follows: platelets $y = 0.0015x^3 - 0.1896x^2 + 8.5114x + 54.199$, $R^2 = 0.9729$; prothrombin time, $y = 9.5166e^{0.017x}$, $R^2 = 0.9823$; aPTT, $y = 18.461e^{0.0239x}$, $R^2 = 0.9793$; Fibrinogen $y = 76.999e^{0.0141x}$ $R^2 = 0.8704$; factor VIII $y = 28.505e^{0.0221x}$ $R^2 = 0.9396$ (Fig. 1).

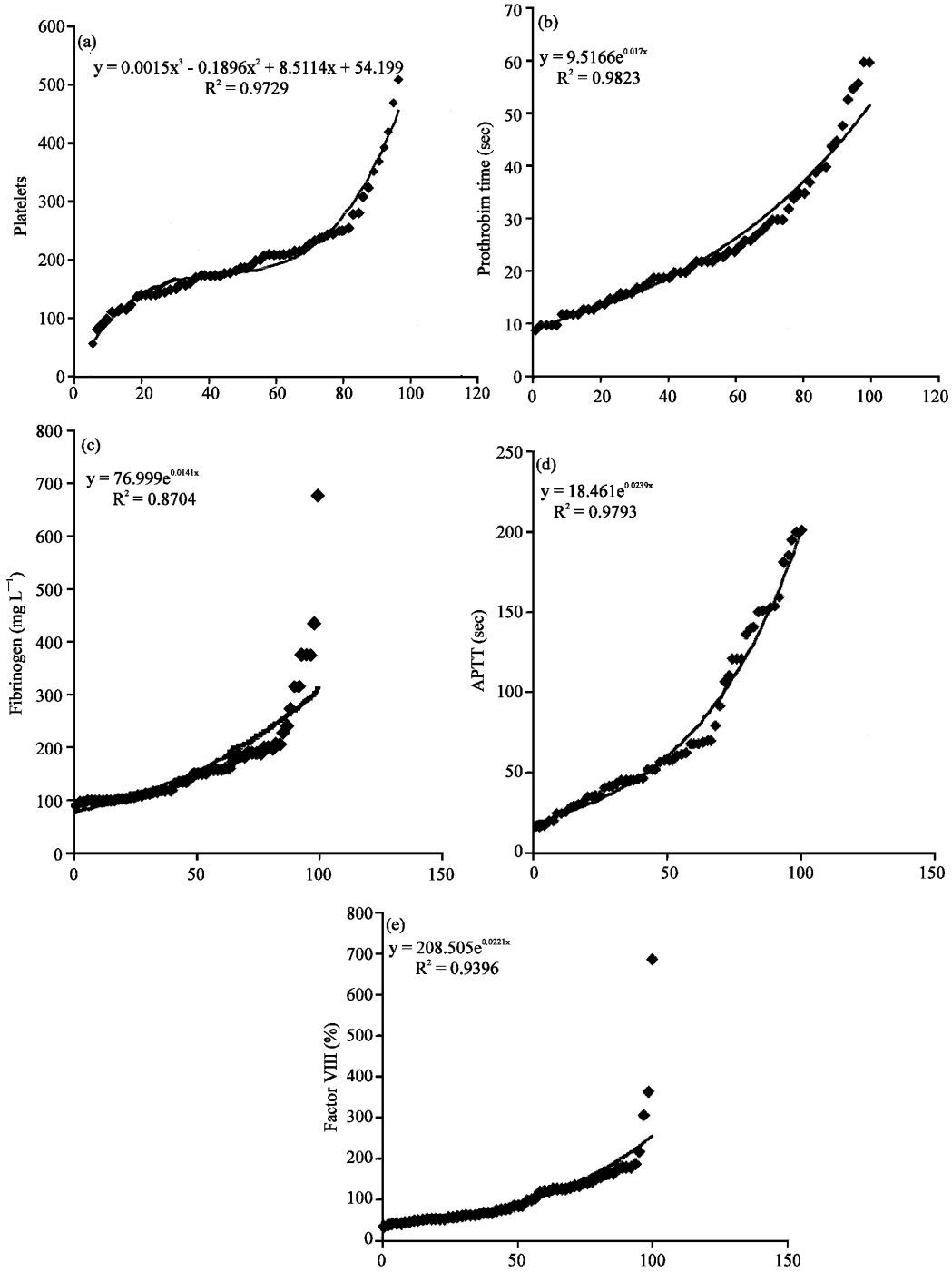

Fig. 1: Regression lines of best fit between the various parameters and trimesters

Table 3: Effect of age on coagulation parameters in pregnancy

	Parameters				
Age-group (years)	Platelet count ($\times 10^9$/L)	Prothrombin time (Sec)	aPTT (Sec)	Fibrinogen mg/100 mL	Factor VIII (%)
≤ 20	-	-	-	-	-
21-25	252.7±140.4	25.7±13.6	73.6±56.70	161.5±89.5	100.7±51.30
26-30	225.1±85.50	25.3±12.7	79.1±49.30	195.6±118	90.1±52.30
31-35	200.3±93.50	22.5±15.0	53.4±27.50	136.6±48.8	93.1±48.70
36.40	199.5±45.70	29.0±16.3	90.3±77.80	146.6±68.7	126.6±121.1
>40	162.5±10.60	29.5±6.30	177.0±25.40	237.6±194.1	496.8±269.8
Duncan test of significance	0.09^{ns}	0.411^{ns}	6.297**	3.174*	7.285**

aPTT = Activated partial thromboplastin time, ns = not significant, * = $p<0.05$, ** $p<0.01$

Table 4: Effect of trimesters on the coagulation parameters

	Parameters				
Trimesters (weaks)	Platelet count ($\times 10^9$/L)	Prothrombin time (Sec)	aPTT (Sec)	Fibrinogen mg/100 mL	Factor VIII (%)
1st (= 12) n = 16	189.2±41.00	27.2±15.1	86.7±61.3	219.7±126.5	75.2±41.00
2nd (13-24) n = 78	237.1±98.70	24.9±13.7	76.3±53.7	178.1±108.2	124.1±118.7
3rd (>25) n = 32	200.2±108.4	25.4±12.0	74.8±51.3	141.0±55.80	89.9±43.70
Duncan test of significance	0.128^{ns}	0.685^{ns}	0.586^{ns}	0.084^{ns}	0.063^{ns}

Table 5: Pearson correlation of all the variables tested among the subjects

	Platelet count ($\times 10^9$/L)	Prothrombin time (Sec)	aPTT (Sec)	Fibrinogen mg/100 mL	Factor VIII (%)
Platelet count	1.000				
PT	-0.111	1.000			
aPTT	-0.060	0.370**	1.000		
Fibrinogen	-0.066	0.036	-0.109	1.000	
Factor VIII	-0.037	0.244*	0.443**	0.073	1.000

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, PT = Prothrombin time, aPTT = Activated partial thromboplastin time

DISCUSSION

Haemostatis is a complex physiological process that leads to the arrest of bleeding and it consists of several components: platelets, plasma proteins and blood vessels and endothelial cells (Mazza, 2004). Normal pregnancy has been reported to be associated with a manifest shift of coagulation and fibrinolytic systems towards hypercoagulability (Holmes and Wallace, 2005; Eichinger *et al*., 1999). A notable finding in this study was that normal pregnancy was associated with significant increase in all coagulation parameters; platelet counts, prothrombin time, activated partial thromboplastin time and fibrinogen factor VIII assay.

The reference ranges for some coagulation parameters in pregnant women have been reported recently by Temal *et al*. (2007). In their study, using 2.5% and 97.5% percentile or 95% percentile as reference value, the prothrombin time test was 23.0-30.8 sec, while fibrinogen was 3.65-9.02 g L^{-1}. In this study, the prothrombin time was 25.3 sec as against 19.9 sec among the non-pregnant controls. This increase was found to be significant and correlated significantly with aPTT and factor VIII. The increase in Prothrombin time is consistent with the findings of Hellgren (2003), in which increased prothrombin time and aPTT was reported. It is also consistent with the finding in this study in respect to fibrinogen concentration. In this study, pregnancy was found to exert significant increase in fibrinogen. The implication of these finding is that pregnancy is associated with change in haemostatic balance in the direction of hypercoagulability (Temal *et al*., 2007; Hellgren, 2003).

With respect to platelets, it has been reported that normal pregnancy may be associated with a lower mean platelet count than non-pregnancy (Burrows and Kelton,1990; Boehlen *et al*., 2000). Approximately 30-50% of cases of severe pre-eclampsia has been reported to be associated with thrombocytopenia (platelet count below 150,000 mm^{-3})(Sharma *et al*., 1999; Leduc *et al*.,1992). The findings in this study are at variance with the earlier reports. The platelet count in this study was significantly higher among pregnant women than the non-pregnant controls. This increase was found to be independent of their ages and trimester. This is contrary to the observation of Hellgren (2003), who reported that in normal pregnancy, even though the platelet count is within normal limit, during the third trimester, benign gestational thrombocyto penia (80-150$\times 10^9$/L) could be observed. Excessive platelet activation by dysfunctional endothelium, as a result of abnormal nitric oxide, prostaglandin and endothelin release and metabolism as

been suggested to account for increased platelet turnover and ultimately reduced numbers (Davies *et al*., 2007).

Advanced maternal age has been reported as a risk factor for pregnancy-related thrombosis (De Sweet, 1996). In this study, mothers of above 40 years demonstrated increased values in aPTT, fibrinogen and factor VIII supporting the earlier studies that advanced maternal age is a risk factor and pregnancy related thrombosis. There was no effect of age on other parameters tested. There was no statistical significant change between trimesters and the variables tested.

To what extent the trimesters influences the coagulation parameters were ascertained using regression analysis to determine the lien of best fit. Platelet was found to relate polynomially with the gestation period (trimesters), while the rest of the parameters demonstrated an exponential relationship with the gestation period (trimesters). Polynormial regression is a parabola, which means values can increase then decrease or decrease then increase. This may explain why platelet values fluctuated within the three trimesters; 189.2±41 in 1st trimester, then 237.1±98.7 in 2nd trimester and then decrease to 200.2±108.4. However, there was no statistically significant difference between platelet count and the various trimesters.

Exponential regression can strickly decrease or strickly increase. It is therefore not surprising that fibrinogen values can fall sharply from 219.7 mg/100 mL in first trimester to 141.0 mg/100 mL in the third trimester even though statistically it was not significant. Similarly, factor VIII could rise sharply from 75% in first trimester to 124% in the second trimester and decrease sharply again to 89.9% by the third trimester. Parameters that demonstrate exponential relationship with gestation should be carefully monitored throughout the period of the pregnancy.

CONCLUSION

We conclude that normal pregnancy state results in the significant increase of most coagulation parameters (especially those tested in this study) and increase maternal age (>40 years) is a risk factor for hypercoagulability.

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