Haemostatic Parameters During Pregnancy In Ilorin, Nigeria

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Abstract

The objective of this study was to document the effect of pregnancy on some haemostatic parameters (Prothrombin time [PT], activated partial thromboplastin time [APTT], fibrinogen concentration, euglobin clot lysis time [ELT], and platelet count) in different trimesters of pregnancy.

One hundred and eighty women with non-complicated pregnancy within the age range of 17-40 years (mean± SD 27.1±3.9years) were recruited for the study and sixty non-pregnant, normotensive apparently healthy age-matched women were used as control. All haemostatic parameters evaluated were determined using standard techniques. Significant differences were observed between the haemostatic parameters studied and controls except for platelet count.

Prothrombin time was significantly shorter ($p<0.001$) and fibrinogen significantly higher ($p<0.001$) as pregnancy advanced but, other parameters were not influenced by the gestational age. There was a significant difference between the fibrinogen level in older subject compared with subject of younger age group with a mean of 5.5g/l and 4.0g/l ($p<0.05$) respectively. There was no significant relationship in the haemostatic parameters with parity and educational level of all the subjects. In conclusion, the results of haemostatic screening tests in this study suggest some degree of activation in pregnant women.

Keywords: Haemostasis, Pregnancy, Nigeria

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Introduction

Pregnancy is accompanied by major changes in the coagulation and fibrinolytic system. There are marked increases in fibrinogen and factor VIII levels, while others such as factors VII, IX, X and XII also increase though to a lesser extent. There are significant alterations in the coagulation and fibrinolytic system during pregnancy and these, together with increase in blood volume and the unique phenomenon of myometrial contraction are thought to help in minimizing the hazard of haemorrhage during and after placental separation. However, these changes also carry the risk of rapid and excessive response to coagulant stimuli.[1]

Howie[2] in 1979 suggested that during normal pregnancy, the concentrations of many of the clotting factors rise, thereby increasing the potential to generate fibrin. There is also evidence of increased thrombin activity during normal pregnancy, which sharply increases during placental separation.[2] Pregnancy is associated with a hypercoagulable state, which becomes more pronounced in the prenatal period.[3] The increased level of fibrinogen and other coagulation factors during pregnancy probably represent a compensatory response to local utilization and the resulting hypercoagulability will be advantageous to meet the sudden demand for haemostatic components at placental separation.[2]

Antithrombin III which is the main inhibitor of thrombin and activated factor X shows no compensatory rise during pregnancy but is increased during the puerperium.[2] However other studies found lower activity during pregnancy.[4,5,6] It was reported by Weenik et al, that the plasma level of antithrombin III is within normal range during normal pregnancy but there is significant reduction of antithrombin III in pregnancy-induced hypertension and the decrease is proportional to the degree of both maternal and fetal morbidity.[7] In association with this, is decrease plasminogen activator activity leading to reduced fibrinolytic activity which remains low but return to normal after placental separation.

The platelet count and function during pregnancy has generated a lot of controversy. Some authors reported no significant change compared with the normal non-pregnant state,[8] while others reported a significant decrease in the numbers of circulating platelets as pregnancy advances.[9] Fay et al showed a significant fall in the platelet count during the last 8

weeks of pregnancy, although the level was still within ıe normal non-pregnant range.[10] In pregnancies ɔmplicated by preeclampsia and intrauterine growth retardation, there is a significant fall in platelet count.[11] Pregnancy, delivery and puerperium are associated ʻith complex and still incompletely understood physiological changes involving the blood coagulation and plasma fibrinolytic system.[12] Understanding of ıese physiologic changes is necessary to manage two ɔf the more serious problems of pregnancy i.e. thrombo-embolism and disseminated intravascular oagulation.

The aim of this study was to determine the effects of pregnancy on some haemostatic parameters of apparently healthy Nigerian pregnant women over a ɔeriod of six months. Owing to paucity of data on the ıaemostatic changes in normal pregnancy amongst the black population especially in Africa, this study will herefore be relevant to establish the values for some ıaemostatic parameters in normal pregnancy in this environment and see how they compare or differ from ɔstablished values in Caucasians.

Patients and methods

Two hundred and forty women which included ɔf 180 pregnant and 60 controls were used for the study. The age range was between 17-40 years mean of 27.1±3.9 years. The study period was between May and October of the same year. Subjects were grouped into the three trimesters of pregnancy. Sixty women with gestational age (GA) 6-13weeks were grouped as first trimester, GA between 14-26 weeks grouped as being in the second trimester while the remaining sixty subjects with GA 27 weeks and above made up those in the 3rd trimester.

The subjects were pregnant women who reported for antenatal booking at the Department of Obstetrics and Gyaenecology of Unilorin Teaching Hospital. The gestational age was determined using subject last menstrual period and Ultrasound estimation. Only subjects who were certified fit based on careful history taking and physical examination were selected for the study .Exclusion criteria included sickle cell anaemia, hypertension, diabetes mellitus,varicose veins, jaundice, use of drugs like aspirin, dicumarol. Sixty age-matched non-pregnant, normotensive women were recruited as control and they included labortatory staff, students of the school of health technology Offa, medical students and student nurses. Their non- pregnant status was confirmed by pregnancy test and exclusion criteria were the same as for the study group. Verbal and written consent were obtained in each case. After informed consent and subject being certified fit to participate in the research, about 7ml of venous blood sample was taken from the antecubital vein mostly between 8-10am. 4.5ml of blood was dispensed into a covered bottle containg 0.5ml of 3.8% trisodium citrate, the sample was mixed thoroughly and centrifuged at 2000g for 10minutes and the plasma separated into another clean tube and used immediately for the coagulation tests or kept on ice until analyzed. The separation of sample was done within 2hours of collection. The remaining 2.5ml was dispensed into a bottle containing ethylene diamine tetra acetic acid as anticoagulant and used for platelet counts. The control blood samples were treated the same way.

The Prothrombin time (PT), Activated partial thromboplastin time (APTT) were estimated using commercially prepared kits from Quimica Clinica Aplicada S.A Ampostal/Spain and procedure based on methods described by Dacie and Lewis.[24] Fibrinogen estimation was carried out by the dry clot weight method of Ingram,[25] while Euglobulin clot lysis time was carried out using calcium chloride in borate buffer,[17] and platelet count was determined from full blood count by automated blood cell counter Sysmex KX 21(Product of Sysmex Corporation Kobe, Japan). Each of the procedures for PT and APTT were carried out on the test and control plasma in duplicate at the same time, and the results were expressed as the mean of the paired values for test and control. The results were analysed by generating mean and frequency distribution for the different variables which were then displayed in tables .Comparisons was made using standard statistical methods in which categorical data was compared by Chi-square and discrete variables by T-test, 95% confidence level was observed.

Results

The means value of PT, APTT, Fibrinogen concentration, ELT and Platelet counts in the subjects were 12.7±1.7seconds, 36.1±5.4seconds, 4.8±1.5g/l, 224.3± 65.9 minutes and 167.6± 63.9×109/l respectively. In the controls group the means of PT, APTT, Fibrinogen, Euglobin clot lysis time and platelets counts were 13.6±1.3seconds, 41.6±5.9 seconds, 3.2±1.1g/l, 192±56.5 minutes and 172.3±66.3 respectively. There was significant difference between the mean values of coagulation parameters in the subjects and that of controls (p=0.001) except platelet count with p value of 0.057 (Table 1).

The PT and fibrinogen concentration were significantly affected by the gestational age of the pregnancy with mean PT of 13.2±1.9seconds, 12.7±1.5 seconds, and 12.1±1.5 seconds and fibrinogen concentration of 4.1±1.5g/l, 5.1±1.7g/l and 5.2±1.3g/l in the 1st ,2nd ,and 3rd trimesters respectively (p value 0.001 and 0.001). However, gestational age of the pregnancy had on significant effect on the APTT,ELT and platelet counts(p value 0.405,0.770 and 0.880) respectively (Table ll).

The effect of age on the haemostatic parameters was only noticed in the fibrinogen as the

Table 1. The mean haemostatic parameters among subjects and controls

Parameter	Subjects(n=180)	Control(n=60)	P value
Prothrombin time(seconds)	12.7±1.7	13.6±1.3	0.001
Activated partial thromboplastin time(seconds)	36.1±5.4	41.6±5.6	0.000
Fibrinogen level g/l	4.8±1.5	3.2±1.1	0.001
Euglobin clot lysis time(minutes)	228.4±65.9	192.9±56.5	0.000
Platelet count $\times 10^9$/l	167±63.7	172.3±66.3	0.057

Normal Reference values[28]: PT 12-14seconds, APTT 30-40 seconds , Fibrinogen 1.5-4.5g/L ELT 90-240 minutes Platelet count 150-400$\times 10^9$/L

Table II: Mean haemostatic parameters of subjects in relation to trimesters

Parameter	1st trimester	2nd trimester	3rd trimester	p-value
Prothrombin time(seconds)	13.2±1.9	12.7±1.5	12.1±1.5	0.000
Activated partial thromboplastin time(seconds)	35.7±6.4	35.6±4.8	36.8±4.9	0.405
Fibrinogen level g/l	4.1±1.5	5.1±1.7	5.2±1.3	0.000
Euglobin clot lysis time(minutes)	225.4±64.9	226.3±73.8	228.4±65.9	0.770
Platelet count$\times 10^9$/l	164.5±64.9	168.0±64.9	170.3±66.9	0.880

Table III : Effect of parity on haemostatic parameters

Parameter	Nulliparous	Multiparous	Grandmultiparous	p-value
Prothrombin time(seconds)	12.8±1.8	12.6±1.7	12.6±1.1	0.820
Activated partial thromboplastin time(seconds)	36.8±5.2	35.6±5.5	36.0±6.5	0.350
Fibrinogen level g/l	4.8±1.8	4.7±1.4	5.1±1.3	0.750
Euglobin clot lysis time(minutes)	223.2±67.9	231.1±66.0	236.0±52.6	0.696
Platelet count$\times 10^9$/l	157.4±59.6	175.6±65.0	167.0±63.7	0.161

mean fibrinogen in the older subjects was 5.5g/l (28-40years) as compared with younger subjects who had mean fibrinogen of 4.0g/l (18-27years). PT, APTT, ELT and Platelets counts were not affected by the age of the subjects (p value of 0.268, 0.799, 0.217 and 0.678) respectively. The subjects parity does not have significant effect on the haemostatic parameters, likewise the level of subjects' education which might be related to their socio- economic status.

Discussion

During normal pregnancy, the haemostatic balance tilts in the direction of hypercoagulability which helps to reduce bleeding complications during delivery.[13] The changes in the coagulation system during normal pregnancy are consistent with a continuing low-grade process of coagulant activity.[13] The hormones which are necessary for the maintenance of pregnancy i.e. estrogen and progesterone increase several folds and these especially estrogen stimulate hepatocytes thereby increasing the production of

virtually all coagulation factors. Progesterone has been found to increases decidual tissue factor and also increase the synthesis of plasminogen activator inhibitor type 1.[14] Elevation of the levels of certain coagulation factors and the fibrinolytic inhibitors occur in practically all healthy pregnant women which is most likely the result of small amounts of procoagulant factors such as tissue thromboplastin which could cause direct and slow systemic activation of the coagulation cascade.[15] In this study, the results of coagulation screening tests that were carried out were in support of a hypercoagulable state in pregnancy. The prothrombin time, which assesses the factors in the extrinsic pathway, was reduced when compared to the value in non-pregnant controls. The findings in the study are also similar to those of Hellgren in 2003, [13] who observed increase in prothrombin complex level (prothrombin time) expressed as international normalized ratio (INR) of less than 0.9. Similarly, Uchikova et al in 2005, [16] reported prothrombin time as being significantly shortened in pregnancy compared with control. By contrast however, the work of Adediran et al in 1999[17] in Ile-Ife, showed prolongation of prothrombin time in the face of what was otherwise a hypercoagulable state. In this study, the mean prothrombin times in the subjects in the 1st, 2nd and 3rd trimesters of pregnancy showed that production of these coagulation factors increases as pregnancy advanced, as there was statistically significant reduction in prothrombin time from the 1st to the 3rd trimesters of pregnancy (P value 0.001). Significant difference was also noticed in the partial thromboplastin time with Kaolin (PTTK) among the pregnant and control subject, which shows that levels of factors in the intrinsic pathway are also increased in normal pregnancy. There was no statistically significant difference in the result of PTTK in various trimesters of pregnancy. This may probably be due to the fact that the estrogen – induced stimulation of factors VIII and IX production is much less than for the extrinsic pathway factors. Furthermore estrogen has less effect in stimulating endothelial cells and macrophages, which are also sites of production and storage of Factor VIII. The fibrinogen level was significantly elevated in normal pregnancy and the level increased as pregnancy advanced. This finding is similar to that of Adediran et al,[17] who found significantly higher value for fibrinogen in normal pregnancy. Similar findings were reported by Hellgren[13] and Kobayashi[18] who concluded that increased fibrinogen was an important factor in pregnancy as it assists in preventing postpartum haemorrhage with 5-10% of the total circulatory fibrinogen being deposited at the placental site. There was a significant difference in the fibrinogen level of women in the older age group compared with those of younger age group ($P<0.05$). The increase in fibrinogen observed with age is well supported by various studies in a number of populations.[19, 20] The increase in fibrinogen with age is thought to be mainly attributable to a slower rate of disposal rather than increased synthesis.[21] From this study, the euglobulin clot lysis time, which is one of the tests of fibrinolysis, was significantly increased in normal pregnancy when compared to non-pregnant control. This finding is similar to that of Adediran et al[17] who found significant increase in euglobulin clot lysis time in normal pregnancy. Wright et al [22] also confirmed a reduction of the fibrinolytic activity of the plasma euglobulin fraction from the second trimester and a parallel reduction in tissue plasminogen activator and increase in tissue plasminogen activator inhibitor activity with rapid return to non-pregnant level postpartum.[24,25] Plasminogen activator inhibitor type–2 which is undetected in non-pregnant control plasma was measurable in the first trimester, increased through pregnancy and remained at a higher concentration postpartum.[22] There was no significant difference between the platelet count in controls and that of the subjects, which is similar to that of Adediran et al [17], who reported no difference in the mean platelet count in the normal pregnant women and non-pregnant controls. However, Bonnar and Syneny both reported reduction in platelet count in pregnancy.[9,10] Other workers however, have documented higher random platelet count in some pregnant and postpartum women than in the non-pregnant state. It has also been suggested that the occurrence of thrombocytopenia in labour or prepartum might be related to the low grade "physiologic DIC" that might accompany normal delivery.[24,1] Although the various physiological changes, which occur in pregnancy, make pregnancy a hypercoagulable state, there is a balance between coagulation and fibrinolytic activities which limits the likelihood of actual thrombosis. Furthermore, changes in the haemostatic system during pregnancy may be more marked in the uteroplacental than in the systemic circulation and thus the pregnant women may be relatively well protected against the thrombotic effects of a hypercoagulable state.

Conclusion

From this study, we have confirmed, as in other reports worldwide the development of a transient hypercoagulable state in normal pregnant women in Nigeria. The evidence for this is found in the significant shortening, compared to the non-pregnant state, of the prothrombin time and the partial thromboplastin time with Kaolin. The increased in plasma fibrinogen and the reduction of fibrinolytic activity as shown by a prolonged euglobulin lysis time. The results obtained from this study have given us a better interpretation of the coagulation screening tests in pregnancy. Results outside the 'normal range' can be closely monitored for

any attendant complications in pregnancy.

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