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Fibrinogen Concentration and Thrombin Levels in Pregnant Women in Nnewi, Anambra State, South, Eastern Nigeria.

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ABSTRAST
This study was aimed at looking at the influence of pregnancy on fibrinogen concentration and thrombin time (TT) levels. A total of 195 pregnant women between 18 and 35 years were recruited, 59 were in their first trimester (group A), 61 second trimester (group B) and 75 the third trimester (group C). A total of 150 apparently healthy non pregnant women were used as normal control (group D). Blood samples which was collected from each of the participants after obtaining informed consent was tested for the fibrinogen concentration using Clauss method and thrombin time using two stage method. The study was approved by Nnamdi Azikiwe University Teaching Hospital Ethical Committee Nnewi. ANOVA and students t-test were used for statistical analysis. The results showed that fibrinogen concentration (mg/dL) were significantly higher ($p<0.05$) in group A, B and C as compared with the control group. TT (s) were significantly higher ($p<0.05$) in group A, B and C compared with the control group. This study therefore suggests that the increase in these coagulation factors observed are due to increased thrombin generation, inflammatory state of pregnancy and fibrinogen being an acute-phase protein. It is important to obtain a baseline of these parameters for all pregnant women during antenatal visits, in order to detect any abnormality early.
Key words: Fibrinogen concentration, thrombin time and pregnancy.

INTRODUCTION

Pregnancy is the carrying of one or more offspring known as foetus or embryo inside the womb of a female. In pregnancy, physiological changes affect coagulation and fibrinolytic system. These physiological changes are seen in blood volumes, blood pressure, gastrointestinal system, metabolism, renal physiology, the endocrine system, haematological parameters, liver enzymes (Jamjute *et al*., 2009). Many clotting factors increase and anticoagulation factors decrease causing augmented coagulation and decrease fibrinolysis (Dahlman *et al.*, 1999; Thorton and Dauglas, 2010). Haemostasis abnormalities have been associated with various complications of pregnancy (Awodu and Enosolease, 2003). These changes result in a state of hypercoagulability and are likely due to hormonal changes and increase the risk of thromboembolism (Salter *et al.*, 2007). These changes in haemostatic system are considered to be in preparation for the maintenance of the placental function which occurs during pregnancy. These substances stimulate clot formation to stop maternal blood loss. As placental blood flow is up to 700ml/min considerable haemorrhage can occur if clotting fails (Dahlman *et al.*, 1999). Increase in the blood level of these coagulation factors is maximal in the 3rd trimester of pregnancy Fibrinogen is a soluble plasma glycoprotein protein produced in the liver by hepatocytes. It is a large molecule, made up of two identical halves, each half composed of three protein chains (α alpha, β beta, and γ gamma). Thrombin cleaves fibrinogen with the release of fibrinopeptides A and B, producing fibrin monomer which then polymerizes and is stabilized by the action of factor XIII, Fibrin is then cross linked by factor XIII to form a clot. In its natural form, fibrinogen can form bridges between platelets, by binding to their GpIIb/IIIa surface membrane proteins. Thrombin is the essential enzyme product of the blood coagulation enzymatic cascade. Thrombin is a "trypsin-like" serine protease protein that in humans is encoded by the F2 gene (Degen and Davie, 1987). Prothrombin (coagulation factor II) is proteolytically cleaved to form thrombin in the first step of the coagulation cascade, which ultimately results in the stemming of blood loss. Thrombin in turn acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzing many other coagulation-related reactions. In the blood coagulation pathway, thrombin acts to convert factor XI to XIa, VIII to VIIIa, V to Va, and fibrinogen to fibrin. In a country like Nigeria, high maternal mortality and fetal wastage is

associated with high incidence of antepartum and postpartum haemorrhage, high parity, home deliveries not supervised by trained personnel, inadequate and inaccessible health facilities and late presentation of cases to health facilities. The aim of this study was to determine to what extent pregnancy affects fibrinogen concentration and thrombin time when compared with a control group of non pregnant women.

SUBJECTS AND METHODS

Subjects: One hundred and ninety five (195) pregnant women aged 18-35 years attending antenatal clinic in Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi between September and November 2011 were screened. The questionnaires were completed and their informed consent was obtained. The information obtained using the questionnaires include age, parity, past history of pregnancy related problem(s), duration of pregnancy, history of any miscarriage. Out of 195 pregnant women recruited, 59 were in the first trimester (group A), 61 second trimester (group B) and 75 third trimesters (group C). One hundred and fifty (150) non pregnant women (group D) were also screened and they served as controls.

One thousand eight hundred milliliter (1.8mls) of venous blood was collected from each of the subjects, and was added into Trisodium Citrate container containing 0.2mls of Trisodium Citrate (1:9 dilution) mixed immediately by reverse uniform inversion for fibrinogen concentration and thrombin time. The sample was spun with a bench centrifuge at 3000rpm for 10mins. Then the clear plasma was collected into a clean dry plastic container. The test was performed using Rayto Semi Auto coagulation analyser RT-2204C model manufactured by Rayto Life and Analytical sciences Co., Ltd.

Exclusion Criteria: Pregnant and control subjects with bleeding disorders, underlying coagulation disorders, pregnancy related problem, patients on anticoagulants therapy and patients that refused to give consent were excluded.

Study Sites: This study was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi with the approval of the Nnamdi Azikiwe University Teaching Hospital Ethical Committee. The samples were collected in the Antenatal Clinic while analysis was carried out in the Hospital Laboratory.

Determination of Thrombin Time (TT): This was done using the Two Stage Method. It measures the time it takes plasma to clot, when thrombin acts upon fibrinogen converting it to insoluble fibrin in the common pathway. The TT reagent was reconstituted with 1ml of distilled water and mixed by inversion and allowed to stand at room temperature. 100µL of sample was added to the test cuvette, and then 50µL of the reconstituted TT reagent was added. The clotting time was recorded in seconds. One normal control plasma sample was setup for each batch of tests.

Determination of Fibrinogen concentration: This was done using the Clauss Method**.** In the presence of high concentration of thrombin, diluted plasma gives a required time of clot formation which is inversely proportional to the fibrinogen concentration. The sample is diluted with 450µL of imidazole buffer to 50µL of sample to give a 1:10 dilution. 200µL of prediluted sample is added to the test cuvette, the sample was incubated for 5mins at 37 ^{O}C. Then 100µL of bovine thrombin is added. The time of clot was recorded in seconds and the concentration in mg/dL.

RESULTS: Table 1 showed comparisons of mean±SD TT (s) and fibrinogen concentration (mg/dL) in pregnant women in first trimester (group A), second trimester (group B) and third trimester (group C), and control non pregnant women (group D). The mean±SD fibrinogen concentration 505.97±178.22 for the pregnant women in the group C respectively compared with that of 404.26±143.92 for group A, 422.33±126.75 for group B and 283.87±98.90 for group D, (in each case) showed higher significant different ($P<0.05$). Also the fibrinogen concentration 404.26±143.92 for pregnant women in group A showed a higher mean difference compared with the mean value 283.87±98.70 in group D ($P<0.05$). Moreover, the mean value fibrinogen concentration 422.33±126.75 for group B compared with 283.87±98.70 in group D was significantly higher ($P<0.05$). However, the comparison in fibrinogen concentration mean value between the pregnant women in the group A and B showed no significant value ($P>0.05$).

The mean±SD TT in pregnant women in the group A 16.79±0.82, B 15.59±1.46, C 16.47±1.32 and control group D 14.87±1.08, compared amongst the group showed significant different in value (F 52.988; $P<0.05$). The within group comparison showed that TT 16.79±0.82 for pregnant women in group A compared with that of group B 15.59±1.46 and group D 14.87±1.08 in each case, showed higher significant difference ($P<0.05$). Also the TT 15.59±1.46 for pregnant women in group B compared with 16.47±1.32 for pregnant women in the group C showed significantly lower difference ($P<0.05$). Moreover, the mean±SD of TT 15.59±1.46 for pregnant women in the group B showed higher significant difference compared with the mean value 14.84±1.08 in group D ($P<0.05$). Furthermore, there was higher significant difference in the mean±SD of TT 16.47±1.32 for pregnant women in the group C compared with that of 14.87±1.07 in group D ($P<0.05$). However, the mean values in TT, compared between the pregnant women in group A and C showed similar mean value ($P>0.05$).

DISCUSSION

Pregnancy is a complex physiological process with many physiological changes as seen in increased coagulation factors and decreased anticoagulation factors. During pregnancy there are significant changes in coagulation in the direction of coagulability, thus decreasing bleeding complications in connection with delivery (Olorunshola *et al*., 2011). Thrombin time obtained in pregnant subject was significantly higher than that obtained in the control subjects. This was also observed in the works of (Ekaterine and Ilija, 2005), the slightly elevated TT was as a result of increased thrombin generation in pregnancy which also accounts for the hypercoagulability state in pregnancy, and also as a result a gross elevation of fibrinogen concentration (Dacie and Lewis, 2002). In our study, there is a significant elevated value of fibrinogen concentration in pregnancy when compared with the non pregnant control subject. Duperray *et al* (1997) reported a significant increase in fibrinogen concentration which was mainly due to the inflammatory state of pregnancy and also fibrinogen being an acute-phase protein. Works of (Duperray *et al*. 1997, Romero *et al*. 2007) saw a significant elevated fibrinogen in pregnancy; this elevation may be as a result of its involvement in both cell–cell interaction and the interaction of cell and extracellular matrix like collagen. This highly elevated fibrinogen concentration was markedly seen in the third trimester.

CONCLUSION

During pregnancy, the activation of coagulation is counterbalanced by haemostatic balance thus decreasing bleeding complication in connection with delivery. Physiological changes in pregnancy affect the coagulation and fibrinolytic system, which responses in preparation for protecting against excessive haemorrhage or blood coagulation at parturition. The results of this research work conclude that there is a significantly higher difference in TT and fibrinogen in pregnancy when compared with non pregnant control women.

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TABLE 1: Mean±SD of TT(s) and fibrinogen concentration (mg/dL), compared among pregnant women in first (group A), second (group B), third trimester (group C) and control non pregnant women (group D).

Group	TT (s)	Fibrinogen (mg/dL)
A (n=59)	16.79± 0.82	404.26± 143.92
B (n=61)	15.59± 1.46	422.33± 126.75
C (n=75)	16.47± 1.32	505.97± 178.22
D (n=150)	14.87± 1.08	283.87± 98.90
F (P) Value	52.988(0.000)	51.720(0.000)
A vs B	0.000	0.878
A vs C	0.414	0.000
A vs D	0.000	0.000
B vs C	0.000	0.002
B vs D	0.000	0.000
C vs D	0.000	0.000

Key:
F (P) Value= Mean±SD of TT and fibrinogen concentration, compared among pregnant women in first, second, third trimester and control non pregnant women (using ANOVA).
A vs B= Mean±SD of TT and fibrinogen concentration, compared between pregnant women in first and second trimester (using student t test).
A vs C= Mean±SD of TT and fibrinogen, compared between pregnant women in first and third trimester (using student t test).
A vs D= Mean±SD of TT and fibrinogen concentration, compared between pregnant women in first trimester and control non pregnant women (using student t test).
B vs C= Mean±SD of TT and fibrinogen concentration, compared between pregnant women in second and third trimester (using student t test).
B vs D= Mean±SD of TT and fibrinogen concentration, compared between pregnant women in second trimester and control non pregnant women (using student t test).
C vs D= Mean±SD of TT and fibrinogen concentration, compared between pregnant women in third trimester and control non pregnant women (using student t test).