ASSESSEMENT OF FIBRINOGEN LEVEL AND THROMBIN TIME IN PREGNANT WOMEN IN SOKOTO

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ABSTRACT
Background: Pregnancy is the fertilization and development of one or more offspring, known as an embryo or foetus in a woman's uterus.
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.
Methods: Fibrinogen concentration (FC) was determined using Clauss technique and Thrombin time was determined using two stage method.
Results: The FC high among the pregnant subjects (2.79 ± 0.78 g/L) compared to the non-pregnant controls (1.37 ± 0.16 g/L). The thrombin time of pregnant women was shorter (14.35 ± 1.69 seconds) compared to that of non-pregnant women (21.84 ± 0.77seconds).The mean of FC and TT of the pregnant subjects was compared based on trimester, the mean fibrinogen concentration was significantly higher (p=0.05) among pregnant women in the third trimester (3.31± 0.56 g/L) compared to the second (2.42± 0.57 g/L) and first trimester (2.19± 0.71 g/L) respectively.
Conclusion: our result showed that pregnancy causes changes in haemostatic parameters fibrinogen and thrombin.
Keywords: Pregnancy, Women, Fibrinogen, Thrombin time.

INTRODUCTION

Coagulation is the process by which blood forms clot. It is an important part of haemostasis; the cessation of blood loss from a damage vessel, where by a damaged blood vessels wall is covered by a platelet and fibrin containing clot to stop bleeding and begin repair of haemorrhage or obstructive clotting (David *et al.,* 2009).

Coagulation process is a complex series of enzymatic reaction involving the proteolytic activation of circulating coagulation factors (zymogens) and activity of cofactors (V, VIII) leading to the production of thrombin which convert soluble plasma fibrinogen into fibrin. The fibrin enmeshes the platelet plug, forming a stable thrombus which prevents further blood loss from the damage vessels (Cheesbrough, 2000).Functional fibrinogen concentration is inversely proportional to the time taken for the clot to form (David *et al.,* 2009). Normal fibrinogen activity usually reflects normal blood clotting ability. Significantly decreased fibrinogen activity may be due to decreased or dysfunctional fibrinogen. Reduced fibrinogen activity and antigen levels may impair the body's ability to form a stable blood clot. Chronically low levels may be related to decreased production due to an inherited condition such as afibrinogenaemia or hypofibrinogenaemia or to an acquired condition such as end-stage liver disease or severe malnutrition. Acutely low levels are often related to consumption of fibrinogen such as may be seen with DIC and abnormal fibrinolysis. Reduced fibrinogen levels may also occur following rapid, large-volume blood transfusions (delusional coagulopathy).

Pregnancy is the fertilization and development of one or more offspring, known as an embryo or foetus, in a woman's uterus.

Normal pregnancy is accompanied by major changes in the haemostatic mechanism especially an increase in the level of coagulation factors (fibrinogen, V, VIII, and X) and a noticeable decrease in fibrinolytic activity (Heuwieser*et al*., 2010). Such changes have been interpreted as a physiological development to provide effective haemostasis and preservation of the maternal blood during parturition (Urasoko *et al.,* 2009).

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 (Buseri*et al.,* 2008). It is a characterized by impressive changes in the activating and inhibitory pathways of coagulation and fibrinolysis resulting in an accelerated, but well balanced, process of thrombin formation and resolution. 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, to coagulant stimuli (Thorton and Dauglas, 2010). Thrombin is an enzyme that presides over the conversion of fibrinogen to fibrin. Thrombin is the essential enzyme product of the blood coagulation enzymatic cascade. It 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 (Pallister and Waston, 2010).

In a country like Nigeria, high maternal mortality and foetal wastage is associated with high incidence of ante partum and post-partum haemorrhage, high parity, high case of unbooked pregnant women turn up in labour without any antenatal care, high patronage of unqualified traditional birth attendants (TBA's), home deliveries not supervised by trained personnel, inadequate and inaccessible health facilities and late presentation of cases to health facilities (Akani*et al*., 2010; Degen and Davie, 1987).

Materials and Methods

Study area

All the samples collected (4.5mls citrated venous blood) were analyzed at the Haematology Department of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto. It is among the Hospitals that were established in May 1980 as a second generation teaching hospitals along with Calabar, Port Harcourt, Ilorin, Maiduguri and Jos. The hospital started operating at the Specialist hospital, Sokoto (UNFPA, 2013)It has witnessed different kinds of transformations in recent years which have translated into the provisions of tertiary health care service to the entire Northwest region.

Sample Size Estimation

Blood samples from pregnant women obtained will be compared to that of non-pregnant women as controls.50% was used as the prevalence (P) due to the fact that no prevalence value was found in previous researches.

Limitation

The major limitation of this study was the willingness of subjects to submit to the research study. This limited the number of pregnant subjects and non-pregnant controls recruited into this study.

Study Design

This is a case control study designed to include 100 pregnant women as the subjects and 50 non-pregnant women as controls.

Eligibility Criteria

All consenting adults (≥ 18 years) pregnant (subjects) and non-pregnant (controls) without any history of bleeding disorders or oral anticoagulants use were excluded from the study. Control participant were non-menopausal and non- menstruating.

Exclusion Criteria

All women who do not meet the eligibility criteria are excluded from this study. Menopausal and menstruating women are also excluded as control. 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.

Sample Collection

For each subject a tourniquet was applied around the arm, the antecubitalfosa was disinfected with cotton wool soaked in methylated spirit. About 4.5mls of venous blood was collected using a 5mls syringe into sodium citrate anticaogulant tube. The blood was centrifuged to obtain citrated plasma. The citrated plasma was used for the determination of fibrinogen and thrombin time.

Methods

Fibrinogen concentration

Principle

During the process of coagulation, the enzyme thrombin converts soluble fibrinogen into insoluble fibrin. The time required for this conversion is proportional to the concentration of fibrinogen in plasma (Ochei and Kolhatkar, 2010).

Procedure

One in ten dilutions (1/10) of the test and standard fibrinogen plasma was made in imidazole buffer, and two hundred microlitres (200µl) of diluted plasma was warmed at 37^0C for two minute, hundred microlitres (100µl) of thrombin reagent was added to the plasma dilutions and the clotting time was then measured. The normal range for fibrinogen using the Clauss technique is: 1.7-4.0g/L.

Thrombin Time Test

Thrombin time is proportional to fibrinogen concentration of the plasma. Two hundred micro liters (200µl) of patient plasma was added to a test tube and warmed at thirty seven degree (37^0c).One hundred micro liters (100µl) of bovine thrombin was added. The thrombin time was recorded using a stopwatch as the time taken to form a clot following the addition of the bovine thrombin. The normal range for Thrombin is 15-23 seconds

Statistical Analysis

Data obtained was entered manually into computer statistical software (SPSS version 20.0). Results were expressed as mean ± standard deviation. Student t-test was used to compare means difference of FIBC and Thrombin between pregnant and non-pregnant women.

RESULTS

The fibrinogen concentration (FC) and thrombin time of 100 consecutively-recruited pregnant women and 50 non-pregnant women (as controls) were studied. The mean age and range of pregnant subjects were aged 25.52 ± 4.92 years and 18 to 34 years. The means of FC and TT of pregnant subjects were observed among the pregnant subjects were 2.788 g/L and 14.3 seconds respectively.

In Table1, we show present the mean Fibrinogen concentration and Thrombin Time of pregnant subjects and the non-pregnant control subjects. The means of FC and TT observed among pregnant subjects were compared against the non-pregnant controls. The FC of pregnant subjects and non-pregnant (2.79 ± 0.78 g/L and 1.37 ± 0.16g/L), respectively and the result was statistically significant ($p \leq 0.0001$). The TT of of pregnant and non- pregnant controls (14.35 ± 1.69 seconds and 21.84 ± 0.77 1.69 Seconds) respectively and the result was also statistically significant ($p \leq 0.001$).

Table 1: Fibrinogen concentration and Thrombin Time of Pregnant and non- pregnant subjects.

Haemostatic Parameter	Pregnant Subjects	Non-Pregnant Controls	T	df	*P-Value*
Fibrinogen (g/L)	2.79 ± 0.78	1.37 ± 0.16*	17.72	114.47	0.001
Thrombin (Seconds)	14.35 ± 1.69	21.84 ± 0.77*	-37.32	146.93	0.001

*statistically significant (p<0.001)

Table 2: Fibrinogen concentration and Thrombin Time of Pregnant Subjects based on trimesters.

Trimester	Number	Fibrinogen (g/L)	Thrombin (Seconds)
1st Trimester	22	2.19 ± 0.71^{a}	15.05 ± 1.5^{b}
2nd Trimester	31	2.42 ± 0.57^{a}	14.23 ± 1.6^{ab}
3rd Trimester	47	3.31 ± 0.56^{b}	14.11 ± 2.0^{a}
P value		0.14	0.78

Values with different superscript[a ,b] and [ab] differ significantly per column (p=0.05)

DISCUSSION

Haemostatic abnormalities have been associated with various complications of pregnancy (Awodu and Enosolease, 2003). This research work was carried out to assess some coagulation parameters; Fibrinogen concentration and Thrombin time of pregnant women in Sokoto North Western Nigeria and compared with non-pregnant control subject. This study present statistically significant increased fibrinogen concentration among pregnant subjects compared to the non-pregnant controls. Our result also is in agreement with previous report by Buseri and colleagues (2008) who indicated that pregnancy exert a significant increase in fibrinogen. Finding from this study is also in agreement with previous reports (Duperray et al., 1997; Hellgren, 2003; Imoru *et al.,* 2007 and Romero *et al.,* 2007;Amilo et al., 2013) which indicated a significant increase in fibrinogen concentration during pregnancy. Pregnancy is known to be a procoagulable state; therefore, it is not surprising that this study and other studies have observed an increase in fibrinogen the precursor of fibrin beginning in early pregnancy (Bonnar *et al.,* 1969; Stirling *et al.,* 1984; and O'Riordan and Higgins). Fibrinogen is an acute-phase protein. The increase in fibrinogen seen among pregnant women may be due to the inflammatory state of pregnancy.This study indicated statistically significant reduced Thrombin time among pregnant subjects compared to the non-pregnant controls. This finding is consistent with previous report by Ekaterine and Ilija (2005) which indicated TT is slightly decreased in pregnancy and that this increase was 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).

It was also observed during the course of this research that trimester affects coagulation. The fibrinogen of pregnant women in the third trimester significantly higher than that of the first and second trimester (p=0.05). This study indicated that the level of fibrinogen rose significantly from the first to the third trimester. This result is consistent with previous report by Cerneca *et al.,*(1997) who reported significant increased fibrinogen concentrations between second and third trimesters. Similarly, Choi (2008) observed a similar increase in fibrinogen concentration from first to third trimester with overwhelmed increase in the third trimester. These changes result in hypercoagulable state and could be due to are hormonal changes leading to increased risk of thromboembolism (Dahlman *et al.,* 1999 Eichinger *et al*., 1999Amilo *et al.,* 2007).

We observed that Thrombin time was higher among pregnant women in the first trimester of pregnancy compared to the second and third trimester. This finding is contrast with a previous report by Amilo *et al.,* (2013) which showed that thrombin level increased significantly in the third trimester when compared to first and second trimester. During pregnancy there are significant changes in coagulation in the direction of hypercoagulable state, thus decreasing bleeding complications in connection with delivery.

RECOMMENDATIONS

Findings from this study are a wakeup call on the need to routinely monitor haemostatic parameters of pregnant women. There is need to develop haemostatic reference intervals for pregnant women in this study area. Current reference intervals being used are generally based on samples from non-pregnant women. This may hinder the accurate diagnosis and treatment of haemostatic disorders during pregnancy. It is recommended that pregnant women with haemostatic complications should be under the management and care of a qualified obstetrician to mitigate the possible negative effect during pregnancy and delivery. There is also the need to provide the necessary facilities and trained Medical Laboratory Scientist for effective diagnosis and monitoring of homeostatic disorders associated with pregnancy.

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