Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0404.47**

EVALUATING PROTHROMBIN TIME (PT) OF PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN PLATEAU STATE SPECIALIST HOSPITAL JOS, NIGERIA

Moses D Lugos, Kakji K John, Umanka Y Polit, Mary-Jane N Ofojekwu, Ogbonnaya U Nnanna, James G Damen

Department of Medical Laboratory Science, Faculty of Medical Sciences, University of Jos, P.M.B 2084 Jos, Plateau State : 930001, Nigeria.

ABSTRACT: Physiological hypercoagulable state has been reported in pregnancy, which is believed to be directed at protecting pregnant women from potential haemorrhage during placentation and post-partum periods. In the current antenatal care, blood coagulation screens are not considered. This study was carried out to determine the Prothrombin Time (PT) of uncomplicated pregnant women to evaluate for the status of extrinsic coagulation pathway during pregnancy. A case-control study was designed comprising of 45 uncomplicated pregnant women; consisting of 15 each in the first, second and third trimester respectively. Twenty-five age-matched healthy non-pregnant women were used as the control population. Both the study and control participants granted oral and written consents to participate in the study. The Quick's one - stage Prothrombin Time test of citrated samples from both the study and control cohorts were carried out in duplicates, and average values (in seconds) were reported. Student t-test analysis revealed a statistically significant decrease (P-value =0.002) in the mean PT of the pregnant women (12.82 seconds with SD of ±1.84) compared to the control group (14.29 seconds with SD of ±1.75). The study also showed a significant gradual decrease in the mean PT as pregnancy progresses toward term as 13.85 ± 1.68, 13.21 ± 1.51 and 11.40 ± 1.47 in the first, second and third trimesters respectively (P- value=0.001). We, therefore, suggest the introduction of routine PT in conjunction with evaluation of the intrinsic coagulation pathway by APTT at antenatal care as a screen to help monitor coagulation disorder in pregnancy.

KEYWORDS: Prothrombin Time, Haemorrhage, Trimesters, Pregnancy and Coagulation.

Corresponding Author: Dr. M D Lugos* Ph.D.
Department of Medical Laboratory Science, Faculty of Medical Sciences, University of Jos, P.M.B 2084 Jos, Plateau State : 930001, Nigeria. Email Address: mlugos2003@yahoo.com.

1. INTRODUCTION

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 [1]. Normal pregnancy is associated with substantial changes in the tissue factor pathway and in the wider haemostatic system [2, 3].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 [4].Hemorrhage occupies an important position in the etiology of maternal mortality and therefore, remains a major problem [5]. Coagulation begins almost immediately after an injury to the blood vessels, which causes damage to the endothelial lining the vessel [6, 7]. Coagulation is the process by which blood forms clot [8]. It is an important part of haemostasis; the cessation of blood loss from a damage vessel, whereby a damaged blood vessel wall is covered by a platelet and fibrin containing clot to stop bleeding and begin repair of haemorrhage or obstructive clotting [9]. 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 [10]. Thrombin is an enzyme that presides over the conversion of fibrinogen to fibrin [11]. 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 [12]. Thrombin, an activated prothrombin, in turn, acts as a serine protease that changes soluble fibrinogen into insoluble strands of fibrin, as well as catalysing many other coagulation-related reactions [13]. The sequence of coagulation process involves the triggering of the coagulation cascade, then a series of serine proteases are sequentially activated, resulting in activating prothrombin to thrombin which converts soluble fibrinogen to the insoluble fibrin clot [14, 15]. 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 child bearing age [1, 16]. Venous thromboembolism is an important cause of maternal morbidity and mortality [17]. Changes in the haemostatic mechanism also involve decreased levels of anticoagulant proteins such as protein C and Protein S as well as enhanced thrombin generation and decreased fibrinolytic activity [18]. In Nigeria, prothrombin time test is not part of routine antenatal investigations. Consequently, no laboratory results that can provide evidences in relation to coagulation patterns of the pregnant woman at term. The study is therefore designed to evaluate the impact of normal uncomplicated pregnancy on Prothrombin Time

(PT). It has provided an opportunity to evaluate changes in Prothrombin time in uncomplicated pregnancy. We also assessed the impact of fish consumption on Prothrombin time in normal pregnancy. It is suggested that information from this study will serve as evidence for proper management of pregnant women in order to abdicates complicatios associated to coagulation abnormalities such as haemorrhage and thrombosis.

2. MATERIALS AND METHODS

Study Area

This study was carried out at Plateau State specialist hospital, Jos. This hospital is a tertiary health facility located in Jos North Local Government Area, Plateau State, North Central Nigeria.

Sample Population

A total 70 women aged 18 – 40 years were recruited into the two cohorts for the study. The first cohort comprised of 45apparently healthy pregnant women attending antenatal clinic at the antenatal unit of Plateau State Specialist Hospital, Jos and the second group is made up of 25 apparently healthy non-pregnant women selected from the general population to serve as a control. Pregnant and non-pregnant women were of age-matched.

Study Eligibility Criteria

All consenting adults (18 - 40 years) pregnant (subjects) and non-pregnant (controls) without any history of bleeding disorders or oral anticoagulants use were included in the study. Control participant were non-menopausal and non- menstruating.

Exclusion Criteria

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

Ethical Clearance/ Informed consent

This study was approved by the plateau state specialist hospital ethical committee, and the informed consent was obtained from all the study participants.

Sample Collection

For each subject a tourniquet was applied around the arm, the antecubital fossa was disinfected with cotton wool soaked in methylated spirit. About 4.5mls of venous blood was collected using a 5mlsyringe into the sodium citrate anticoagulated tube. The blood was centrifuged to obtain citrated plasma. The citrated plasma was used for the determination ofProthrombin Time.

Laboratory methodology

The Quick's one-stage technique for PT was used for this assay. Commercial tissue thromboplastin was prepared from rabbit brain or rabbit lung. The Prothrombin Time (PT) appraises the effectiveness of the extrinsic and common pathways of blood coagulation cascade [19, 20].

Prothrombin Time test

The manual method based on Clinical and Laboratory Standards Institute (CLSI) H21 was the

method of choice. Briefly; prior the experiments, working reagents and citrated plasma samples were pre-warmed in a water bath at 37^{o}C. After a gentle mix, 200µL of working reagent was pipetted into the coagulation tube. This was further incubated for 5 minutes at 37^{o}C. After 5 minutes of incubation, 100µL of citrated plasma was added to tubes and a stopwatch was started immediately. At the sight of a fibrin clot, the stopwatch was stopped and coagulation time determined and recorded in seconds. Patients and control samples were carried out in duplicates and the average time (in seconds) was reported as prothrombin time (PT) of the patient. Normal range: 11-16 seconds [21].

Statistical Analysis

The data was presentedon tables and charts. The analysis was carried out using statistical package for social sciences (SPSS version 23.0 software). In all cases P. value of equal or less than 0.05 was taken as statistically significant while P. value of greater than 0.05 was taken as statistically not significant.

3. RESULTS AND DISCUSSION

Data collected from both subjects and controls were analyzed using Statistical Package for Social Sciences (SPSS version 23.0 software). (P-value $\leq$ 0.05) were considered statistically significant while (P-value >0.05) were considered not statistically significant. Figure 1 is a pie chart showing the percentage distribution of the study population. A total of 70 (100%) participants comprising of 45 (64.29%) normal uncomplicated pregnant women and 25 (35.71%) apparently healthy non-pregnant women were used as study population and control groups respectively. The bar chart in figure 2 shows the distribution and rate of fish consumption among study participants and control group. It was observed and presented on the chart that more of the control group (about 58%) ate fish twice each week while those that ate once per week and none per week presents approximately 40% and 5% respectively. For the test group about 63%, 18%, 5% and 5% were found to eat fish twice/week, once/week, daily and none respectively. Table 1 shows the mean PT(s) of the study subjects and control group. A statistically significant decrease in the average PT among pregnant women (12.82±1.84) compared to the control (14.29±1.75) was observed (p-value 0.002). Changes in PT(s) according to trimesters among pregnant women (test group) are presented in Table 2. The result shows a significant decrease in the average PT(s) among pregnant women as pregnancy progresses from first (13.85 ± 1.68), second (13.21 ± 1.51) and third trimester (11.40 ± 1.7) respectively (p-value = 0.001). Table 3 shows the mean PT(s) among pregnant women in relation to the rate of fish consumption. The PT Mean ± SD for None, Once, Twice and Daily fish consumptions per week are 13.26±1.63, 12.37±1.74, 14.55±1.24 and13.48±2.59 respectively. The p-value 0.096 indicates that there is no significant difference due to the rate of fish consumption.

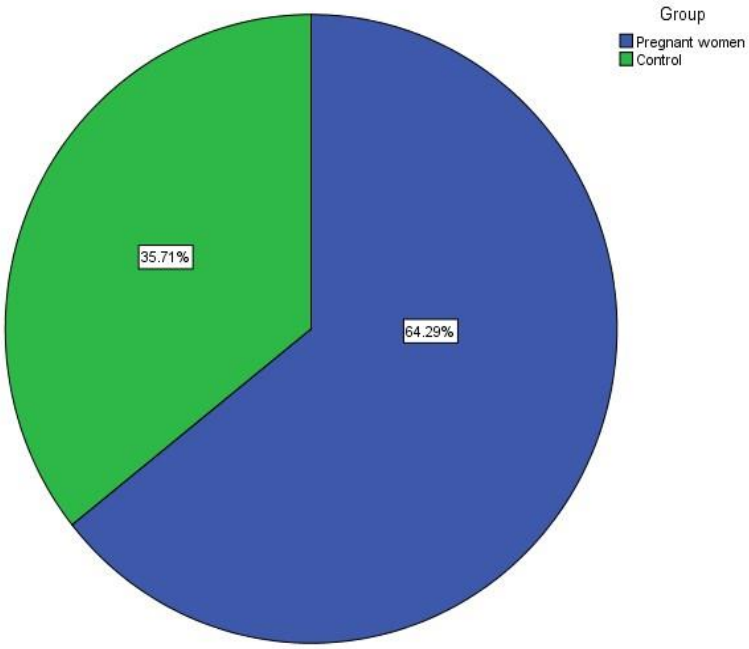

Figure 1: Pie chart showing the percentage distribution of study participants

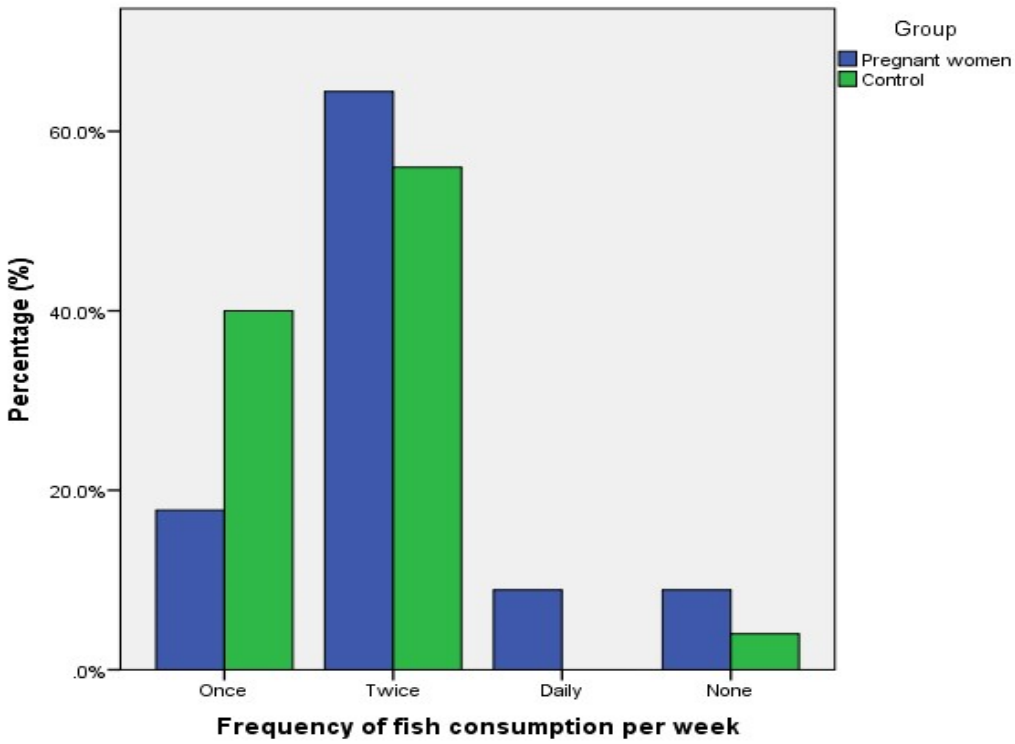

Figure 2: Bar chart showing distribution of study participants according to fish consumption per week

Table 1: Prothrombin time between pregnant and non-pregnant women

Study group	No	Mean ± SD PT	t-test	P value
Pregnant women	45	12.82±1.84	3.261	0.002
Non pregnant women	25	14.29±1.75		

Table 2: Changes in Prothrombin time according to trimesters among pregnant women

Trimester	No	Mean ± SD PT	F-test	P value
First	15	13.85±1.68	10.006	0.001
Second	15	13.21±1.51		
Third	15	11.40±1.47		

Table 3: Prothrombin time according to fish consumption per week among pregnant women

Fish consumption per week	No	Mean ± SD PT	F-test	P value
None	8	13.26±1.64	2.261	0.096
Once	29	12.37±1.74		
Twice	4	14.55±1.24		
Daily	4	13.48±2.59		

DISCUSSION

This research work is design to test the hypothesis of difference which states that pregnancy impact on Prothrombin Time (PT); and the hypothesis of no difference which says that pregnancy does not impact on PT. To test this hypothesis, a total of 70 women were recruited into the study: average PT of 45 pregnant women (test group) was determined and compared with the average PT of the control group (25 non-pregnant women).Table 1 presented the mean PT(s) of the subjects and the control group. Data on Table 1 shows that there is statistically significant difference in the mean PT of pregnant women which was found to be 12.82 ± 1.84 when compared to the control group which was found to be 14.29± 1.75 with P. value 0.002. The result agreed with the hypothesis that pregnancy impact on PT. This has been explained be Durotoye*et al* six years ago that the hormones estrogen and progesterone which are necessary for the maintenance of pregnancy increase several folds and these especially estrogen stimulates hepatocytes (liver cells) thereby increasing the production of virtually all the coagulation factors thus, shortening the PT(s) in pregnant women

[22].Also, it is reported that haemostatic balance tilts in the direction of hypercoagulability in pregnancy and that this is to reduce bleeding complications during pregnancy [23]. In line with this result [22]; [24] and [25] all reported in their separate studies that there was statistically significant difference in the mean PT(s) of normal uncomplicated pregnant women compared to apparently healthy non-pregnant women. Contrary to the result recorded by Cerneca in 1997 that there is no statistically significant association in the mean Prothrombin Time(s) among pregnant women when compared to the control group of non-pregnant women [26]. The result of findings on the changes in PT(s) according to trimesters among pregnant women was also evaluated and presented on table 2. Data recorded on the table shows that in the first and second trimesters mean PT(s) was found to slightly decrease from 13.85 ± 1.68 to 13.21 ± 1.51which was not statistically significant but as the pregnancy progresses to third trimester a statistically significant difference was recorded with the third trimester recording a decrease PT(s) of 11.40 ±1.47.This has been explained that haemostatic balance tilts more in the direction of hypercoagulability as pregnancy advances to help reduce bleeding complications during delivery [23]. Food intake (fish) of the test group was considered and evaluated. The data is presentedin table 3. The result showed that higher number (29) of the women ate fish once per week while the least number (8), 4 ate twice and 4 daily. Fish intake was found to have no statistically significant (P. value 0.096) impact on the mean PT of the subject. Although P. value 0.096 is considered not significant it is on the borderline and an increase in sample size could probably shift it to a considerablevalue.

4. CONCLUSION

The study shows that decreased Prothrombin time during normalpregnancy when compared with control groups of non-pregnancies and also prothrombin time decreases significantly as the pregnancy progresses towards term, although the mean PT in both subjects and controls fell within the normal values. This study agrees with other previous studies conducted on the same population indicating hyper coagulation activity during pregnancy as a complementary mechanism in protecting the mothers at delivery. Fish consumption has no impact on the prothrombin time as shown by the study. It is therefore recommended that Prothrombin time test should be included as part of routine antenatal Haematology test. This will help provide evidence-based data for proper management of pregnant with regard to coagulation disorders associated with pregnancy such as bleeding and thrombosis. Further studies on the impact of fish consumption on PT should be carried out with more sample size.

ACKNOWLEDGEMENT

We are profoundly indebted to our study participants who graciously consented to participate in the study. We are also thankful to Mr. Jalo Philip for helping with the data analysis.

CONFLICT OF INTEREST

We have no conflicts of interest to disclose.

REFERENCES

1. Holmes VA, and Wallace JM. Haemostasis in normal pregnancy: a balancing act? Biochem Soc Trans. 2005; 33(P2); 428-432.
2. Buseri FI, Jeremiah ZA and Kalio FG. Influence of pregnancy and gestation period on some coagulation parameters among Nigerian antenatal women. Res J Med Sci, 2008. 2(6): 275-281.
3. Brenner B. Haemostatic changes in pregnancy. Thromb Res. 2004; 114(5-6): 409-414.
4. Srimala P, Khan I, and Hari PS. Estimation of prothrombin time in pregnancy compared with normal controls. J Evol Med & Dent Sci. 2013; 2(2):72-78.
5. Vora KS, Mavalankar DV, Ramani KV, Upadhyaya M, Sharma B, Iyengar S, Gupta V, Iyengar K. Maternal health situation in India: a case study. J Health Popul Nutr. 2009; 27(2):184-201.
6. Tocantins LM. The mechanism of hemostasis. Ann Surg. 1947; 125(3):292-310.
7. Li J, Chen J, and Kirsner R. Pathophysiology of acute wound healing. Clin Dermatol. 2007; 25(1):9-18.
8. Monroe DM, and Hoffman M. What does it take to make the perfect clot? Arterioscler Thromb Vasc Biol. 2006; 26(1):41-48.
9. Nigel K, Makris M, and Denise OSG. Practical hemostasis and thrombosis. WILEY—BLACKEI I; 2009.
10. Danckward S, Hentze MW, and Kulozik AE. Pathologies at the nexus of blood coagulation and inflammation: thrombin in hemostasis, cancer, and beyond. J Mol Med, 2013. 91(11): 1257-1271.
11. Petersen MA, Ryu JK, and Akassoglou K. Fibrinogen in neurological diseases: mechanisms, imaging and therapeutics. 2018; Nat Rev Neurosci; 19(5): 283–301.
12. Degen SJ, and Davie EW. Nucleotide sequence of the gene for human prothrombin. Biochemistry. 1987; 26(19):6165-6177.
13. Thangadurai A, Minu M, Wakode S, Agrawal S, and Narasimhan B. Indazole: a medicinally important heterocyclic moiety. Med Chem Res. 2012; 21(7): 1509–1523.
14. Norris LA. Blood coagulation. Best Practice & Res Clin Obst & Gyn. 2003; 17(3): 369-383.
15. Lippi G, Franchini M, and Targher G. Arterial thrombus formation in cardiovascular disease. Nat Rev Cardiol. 2011; 8: 502–512.
16. Sultan AA, Tata LJ, West J, Fiaschi L, KM, Fleming KM. Nelson-Piercy C, and Grainge MJ. Risk factors for first venous thromboembolism around pregnancy: a population based cohort study from the United Kingdom. Blood. 2013; 121(19):3953-3961.
17. Awodu OA, and Enosolease ME. Activated Thromboplastin Time In Women With Recurrent Spontaneous Abortions. Ann Biomedical Sci. 2003; 2(1): 42-46.
18. Uchikova EH, and Ledjev II. Changes in haemostasis during normal pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2005; 119(2):185-188.

19. Hjort PF, Rapaport SI, and Owren PA. A simple, specific one-stage prothrombin assay using Russell's viper venom in cephalin suspension. J Lab Clin Med. 1955; 46(1): 89-97.
20. Caldwell SH, Hoffman M, Lisman T, Macik BG, Northup PG, Reddy KR, Tripodi A, and Sanyal AJ. Coagulation disorders and hemostasis in liver disease: Pathophysiology and critical assessment of current management. Hepatology, 2006; 44(4): 1039-1046.
21. Raijmakers MTM, Menting CHF, Vader HL, and van der Graaf F. Collection of Blood Specimens by Venipuncture for Plasma-Based Coagulation Assays: Necessity of a Discard Tube. Am J Clin Pathology. 2010; 133(2): 331–335.
22. Durotoye IA, Babatunde AS, Olawumi HO, Olatunji PO, and Adewuyi JO. Haemostatic Parameters during Pregnancy in Ilorin, Nigeria. Trop J of Health Sci, 2012; 19(2): 18-22.
23. Akinlaja O. Hematological Changes in Pregnancy - The Preparation for Intrapartum Blood Loss. Obstet Gynecol Int J. 2016; 4(3): 1-5.
24. Hellgren M. Hemostasis during Normal Pregnancy and Puerperium. Sem. Throm. Hem. 2003; 29(2): 125-130.
25. Avwioro OG, Ezeobi JO, Oduola T, and Fakunle OO. Prothrombin time and Activated Partial Thromboplastin time in Pregnant Women in Southern Nigeria. J Applied Pharm Sci. 2013; 3(06):179-181.
26. Cerneca F, Ricci G, Simeone R, Malisano M, Alberico S, and Guaschino S. Coagulation and fibrinolysis changes in normal pregnancy. Increased levels of procoagulants and reduced levels of inhibitors during pregnancy induce a hypercoagulable state, combined with a reactive fibrinolysis. Eur J Obstet Gynecology Reprod Biol. 1997; 73(1): 31–36.