Original Article

Increased Prevalence of Activated Protein C Resistance During Pregnancy may Implicate Venous Thrombo Embolic Disorders as a Common Cause of Maternal Mortality in Nigeria

Ukamaka Theresa Nwagha, Uchenna Ifeanyi Nwagha[1], Obike Godswill Ibegbulam, Sunday Ocheni, Iheanyi Okpala, Paul Olisaemeka Ezeonu[2], Onyebuchi Azubuike[2]

Departments of Haematology & Immunology and [1]Obstertics & Gynaecology, College of Medicine, University of Nigeria Enugu Campus, University of Nigeria Teaching Hospital, Ituku Ozalla, Nigeria, [2]Department of Obstetrics and Gynaecology, Federal Teaching Hospital, Abakiliki, Nigeria

ABSTRACT

Background: Acquired resistance to protein C in pregnancy has been established as one of the factors associated with thromboembolic phenomenon, an important cause of maternal mortality and morbidity. **Objectives:** To establish the mean levels of PCA ratio (measure of protein C resistance) of among our pregnant women since maternal mortality rate of the country is on the increase despite efforts to reduce this trend. **Materials and Methods:** A prospective study was carried out in a tertiary institution in Enugu State, Southeastern Nigeria over the 7 months period from May 2010 to November 2010. Two hundred pregnant women and 50 non pregnant female controls were recruited and PCA ratio, (coagulometric assay) were determined. **Results:** There was a non significant difference between the mean and standard deviation PCA ratio of the female non pregnant controls and pregnant women in 2nd trimester 4.32±0.4 and 4.30±0.4 respectively. A significant difference was noted between the controls and pregnant women in 3rd trimester 4.32±0.4 and 3.87±0.5 respectively also between the pregnant women in their 2nd and 3rd trimester 4.30±0.4 and 3.87±0.5 respectively. **Conclusion:** There is increased protein resistance C in our pregnant women. This may implicate thromboembolic disorders as one of the leading causes of increase maternal mortality despite a downward trend in the prevalence of post partum haemorrhage.

KEY WORDS: Acquired protein C resistance, maternal mortality, pregnancy, post partum hemorrhage, thromboembolic disorders

INTRODUCTION

Hemostasis heralds the healing process following tissue injury. It is the process of formation of a fibrin clot with subsequent dissolution of the clot. It is a dynamic process, whereby blood coagulation is initiated and terminated in a rapid and regulated manner.[1] The process of blood coagulation is initiated when sub- endothelial tissue factor is exposed to the blood flow, following either injury or activation of the endothelium.[2]

A variety of inhibitory system, which inactivates either serine proteases or cofactors, inhibits the coagulation process. A dynamic inhibitory system is generated when thrombin binds to its co-factor thrombomodulin, which is constitutively present on the vasculature, and activates protein C to a serine protease activated protein C.[3] Activated protein C inhibits the coagulation reaction by the proteolytic cleavages and concomitant inactivation of factor V, factor Va, factor VIII, and factor VIIIa.[4,5]

Normal pregnancy is associated with significant alterations in all aspects of the Virchow triad: Venous stasis, endothelial damage, and enhanced coagulation, thereby shifting the equilibrium towards the pro-thrombotic state.[6] These include increasing concentrations of most clotting factors, decreasing concentrations of some of the natural anticoagulants, and reducing fibrinolytic activity. Indeed, there is a significant decrease in protein S activity and progressive increase in resistance to activated protein C in 2nd and 3rd trimesters.[7] Consequently, the overall balance of hemostasis tilts towards apparent hyper coagulability as pregnancy progresses even

Access this article online	
Quick Response Code	Website: www.jbcrs.org
	DOI: 10.4103/2278-960X.104291

Address for correspondence
Dr. Ukamaka Theresa Nwagha,
Department of Haematology and Immunology, College of Medicine,
University of Nigeria Enugu Campus, University of Nigeria Teaching Hospital
Ituku Ozalla, Nigeria.
E-mail: theresa.nwagha@unn.edu.ng

up to pueperium.[8] Hypercoagulability in pregnancy is critically, essential, for the provision of adequate hemostasis to the placental site and certainly acts in synergy with uterine contractions to prevent post-postpartum hemorrahage and avert maternal death.

In Nigeria and indeed most developing countries, maternal mortality ratio has remained unacceptably high, despite concerted efforts to curb the trend.[9,10] Several studies from different parts of Nigeria have reported conflicting figures, ranging from 532-2989 per 100000 live births.[9-15] These studies are, however, flawed as they are hospital-based and, therefore, does not adequately represent the entire population. An effort to undertake a multicenter research in institutions located at different socio-economic settings observed that the maternal mortality ratio and their causes varied considerably from institution to institution.[10] Although postpartum hemorrhage has been taunted as the leading cause of maternal deaths,[9,11-13] studies are beginning to incorporate other factors as competing contenders.[10,16-18]

With these obvious discrepancies, one wonders if it still appropriate to expend all energy and resources on PPH at the expense of other potentially fatal determinants of maternal deaths. It is undisputable that efforts to reduce maternal deaths from PPH is yielding positive result as maternal deaths from PPH are abating.[19-21] However, the overall maternal mortality rate in Nigeria is still embarrassingly high.[21,22] The only plausible explanation is the existence of a hidden, undiagnosed or under-diagnosed contributor to maternal deaths, which had been overshadowed by excessive, but deserved attention to PPH. In Nigeria and indeed many developing countries, many unexplained and unexpected, non hemorrhagic maternal deaths occur regularly. These deaths are sparingly reported, and even when reported, autopsies are rarely done. It is extremely likely that most of the death result from thrombo-embolic disease. Unfortunately, this etiological factor is usually placed at the bottom of the list of the common causes of maternal mortality in Nigeria[9-15] and hence, became a negligible factor in obstetric management. Indeed, thrombo prophylaxis is hardly ever contemplated, even with the existence of overt predisposing factors. This is in contrast to events in most developed countries where changes, which occur in the activity of protein C during pregnancy, has been documented to contribute significantly as a leading cause of thrombo-embolic related mortality and morbidity.[7]

As the deadline for the MDGs is fast approaching, and as the menace of PPH is being tackled, thrombo embolic disorders may begin to emerge as a leading cause of maternal deaths in Nigeria. Unfortunately, we are poorly equipped to perform proactively. It is, therefore, expedient to establish factual knowledge if this emerging threat must be confronted. The general thinking that thrombo-embolic maternal deaths are uncommon[10-13] in our environment must be dispelled, and the real danger must be exposed.

Although some Nigerian studies have examined different aspects of coagulation profile in pregnancy,[23-27] there has been none done on protein C activity in pregnancy. Consequently, in order to inform our people and raise awareness on the implication of this emergent variable in the etiology of maternal deaths, a major determinate of coagulability; the protein C activity profile is determined.

MATERIALS AND METHODS

Study area

The study was done in Enugu, the capital of Enugu state in Southeast Nigeria, between May and November 2010. The city of Enugu is situated at about 230 m above sea level. The population of the city is about 464,514, of which, 52.1% are female. Enugu state has a crude birth rate of 45 per 1000, crude death rate of 18 per 1000 of the population and a life expectancy of 51 years.[28] The maternal mortality rate ranges between 750 and 850 per 100,000 live births.[9,29]

Study site

University of Nigeria teaching Hospital (UNTH) antenatal clinics is run every working day, Monday to Friday. Patients are usually seen at booking every 4 weeks till 28 weeks, fortnightly till 36 weeks, and then, weekly till delivery. At booking, obstetric, medical, and surgical history is obtained. Gestational age is estimated from the first day of the last normal menstrual period and is collaborated with ultrasonography. The 1st trimester ultrasound, if available at booking, is preferred. Trimester is defined as the 1st trimester (<14 weeks); 2nd (14 – 27 weeks), and 3rd (>27 weeks). Height and weight are measured, and the body mass index calculated. General, systemic, and obstetrics physical examinations are done. The following routine investigations are also done; hemoglobin, urinalysis, blood group, hemoglobin electrophoresis, fasting blood sugar, ultrasound assessment, and screening for syphilis, HIV, hepatitis B and C.

Sample size calculation

To calculate the minimum sample size (n) for comparing the means of APC activity, the following formula[30]

$$n = \frac{2\,(Z_{\alpha} + Z)^{[2]}\sigma^{[2]}}{\delta^{[2]}}$$

where n=minimum sample size for each group

Z_{α}=% point of the normal distribution corresponding to the two–sided significance level (e.g. if the significance level is 5%, then Z_{α}=1.96)

Z=one-sided percentage point of the normal distribution; corresponding to the power. If the power is 80%,, then Z -= 0.84.

σ=Population Standard deviation

$\delta = m_2 - m_1$=expected difference in means

No such study has been carried out in our population; however, in a pilot study conducted at the commencement of this study using 50 gestational age matched subjects, the largest estimated true difference between the means was 0.4 while the widest population standard deviation was 0.5, therefore, applying the formula

$$n = \frac{2(1.96+0.84)^{[2]}0.5^{[2]}}{0.4^{[2]}}$$

n=25 for each group making a total of 75

In order to make provision for attrition and enhance the power of the study, 200 pregnant and 50 non-pregnant controls were recruited.

Clinical methods

This was a longitudinal study with a control arm involving 200 normal pregnant women between the ages of 16-48 years. Fifty (50) nonpregnant age matched controls were recruited from medical students and hospital staff. After obtaining an ethical approval from the UNTH ethical approval committee, and informed written consent, subjects who met the inclusion criteria were randomly recruited from the antenatal clinic by a simple random sampling technique. They were recruited in the 2nd trimester and were followed up to the 3rd trimester. Women in the 1st trimester were excluded because majorities of our pregnant women book late for antenatal care.[31-33] History, physical examination, and laboratory analysis were performed as earlier stated.

Inclusion criteria

- All normal pregnant women in the 2nd trimester who gave an informed consent

Exclusion criteria

- Women with consecutive abortions before the index pregnancy
- Past medical history with underlying pathologies such as liver disease, inherited bleeding disorders, renal disease, cardiovascular diseases, diabetes, and hypertension
- Pregnant women who smoke
- Pregnant women who consume excessive alcohol (more than 1 pint of beer/day)[32]
- Those already on anticoagulants
- Complicated pregnancies (hemorrhage during pregnancy, pre-eclampsia, gestational diabetes, and multiple pregnancy.

Laboratory procedure

Venous samples were obtained using a 21G needle attached to a 5 mls syringe; 4.5 mls of blood were drawn and put into a sample bottle containing 0.5 mls of 3.2% of sodium citrate, (9 parts of blood to 1 part of citrate). The blood sample was gently but well-mixed. For the control subjects, samples were collected on the 7th day of the menstrual period and analyzed after a negative serum beta HCG pregnancy test.

A 15-minute double centrifugation was done within one hour of sample collection. First, the citrated blood was centrifuged at 150-200 gravitational force for 15 min; a plastic transfer pipette was used to remove the supernatant platelet rich plasma, which was placed in a second plastic tube and re-centrifuged this time at 3000 gravitational under identical conditions. The resulting platelet poor plasma (PPP) was either tested immediately or stored in aliquots at in a -80°C freezer until testing.

Protein C activity was measured using a clot-based assay protein C activator (PCA) assay kit (Diagen UK lot no. 82, 83). Reagents were reconstituted, and assay procedure carried out according to manufacturer instructions. Clot detection was done using a KH202 coagulometer (Jinan Kinghawk Technology, China 2006-2008). This procedure measures the standard Activated Partial Thromboplastic Time (APTT) and then the protein C activator/APTT ratio (PCA/APTT). The ratio of PCA/APTT and the standard APTT gave the PCA ratio. The PCA ratio is a measure for APC resistance. Reference control plasmas, Diagen APC resistance plasma, (lot number 117) were used to validate the test.

Quality control

The samples were tested in batches. Each batch was controlled using APC-resistant plasma. These readings are shown in Table 1 and Figure 1. The mean values of PCA: APTT/APTT was recorded in seconds. The cutoff ratio, used to determine increased protein C resistance for this study, was a value of less than or equal to 4.0 seconds. This value was determined in the hematology laboratory of our institution. A delta check, which is the difference between duplicate laboratory results, which exceeds a predefined limit, was applied. A value of 0.5 was allowed between duplicates results.

Statistical analysis

Data obtained in this study was analyzed using the Statistical Package for Social Sciences (SPSS) version 11, Graph Pad Prism version 5.02, and Graph Pad Prism Stat mate version 2.00. Graph Pad Stat mate version 2 was used to justify the power of the study and to ensure that it is above 80%, which

is the minimum required power for clinical studies. Values were recorded in percentages and mean±standard deviation where applicable. For the numerical data, the one way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test was used to test for significant differences between the groups. The PCA ratio in pregnancy was compared with the values found in the non-pregnant female controls. Chi-square was used to test for significance for the non-numerical data. Values of $P<0.05$ were considered significant at 95% confidence interval.

RESULTS

Out of the 200 pregnant subjects recruited over a 7 month period, 145 completed the survey while a total of 55 pregnant subjects were lost to follow up due to change in locations, opting for care in other nearby hospitals and withdrawal from the study. The resultant compliance rate of 73% (145/200) was recorded. Table 2 shows the value of some socio-demographic characteristics. These include age, parity, weight, height, body mass index (BMI), and the mean gestational age.

Activated protein C APC sensitivity ratio or protein C activity

The Protein C Activity (PCA) was calculated using the PCA. APTT/ordinary APTT as shown in Table 3.

The mean APC ratio of the female non-pregnant controls was 4.32 (0.43). The pregnant subject had a mean APC ratio of 4.27 (0.44) in the 2nd trimester and 3.87 (0.50) in the 3rd trimester. These changes were statistically significant, ($P<0.0001$). However, the post hoc multiple comparisons showed that these significant differences were primarily due to differences between the control subjects versus pregnant subjects in their 3rd trimester and also due to differences in the pregnant subjects between the 2nd trimester versus 3rd trimester ($P<0.001$). However, the differences between control subjects versus the pregnant subjects in their 2nd trimester of pregnancy did not contribute to the significant change that was observed ($P=0.368$). The trimester related trend in PCA is highlighted in Figure 2.

Table 4 shows the percentage distribution of APC ratio.

Table 1: Quality control reference figures obtained during the study

Test	Mean values	SD	CV
APC resistant plasma	78.2	0.16	0.21

Table 2: Some demographic and anthropometric characteristics of the test and control subjects

Variables	Control	2nd trimester	3rd trimester
Age (years)	23.92 (5.96)	26.68 (3.98)	26.68 (3.98)
Height (m)	1.63 (0.06)	1.57 (0.14)	1.57 (0.14)
Weight (Kg)	62.22 (8.99)	71.60 (14.52)	73.93 (11.86)
BMI (kg/m$^{[2]}$)	23.19 (3.08)	29.32 (6.69)	32.67 (12.73)
Parity	0.34 (1.12)	2.50 (1.62)	2.50 (1.62)
Gestational age (Wks)	–	21.2 (3.8)	32.0 (2.9)

M = Meter, Kg = Kilogram, Wks = Weeks

Table 3: Activated protein C APC sensitivity ratio or Protein C activity as calculated by PCA. APTT/ordinary APTT

Test	Control group	2nd trimester	3rd trimester	*P* value
APC/aPTT (secs)	158.60 (13.80)[a]	155.90±6.80[b]	150.80 (9.60)[c]	0.001
aPTT (secs)	39.71 (4.17)[d]	36.83±3.61[e]	39.51 (4.40)[f]	0.001
APC Ratio	4.32 (0.43)[g]	4.27±0.44[h]	3.87 (0.50)[i]	0.001

ANOVA and post hoc multiple comparisons. *a vs. b vs. c ($P<0.001$); a vs. b ($P=0.003$); b vs. c ($P<0.001$); a vs. c ($P<0.001$). †d vs. e vs. f ($P<0.001$); d vs. e ($P<0.001$); e vs. f ($P<0.001$); d vs. f ($P=0.692$). ‡g vs. h vs. i ($P<0.001$); g vs. h ($P=0.368$); g vs. i ($P<0.001$); h vs. i ($P<0.001$). §$P>0.05$=not significant

Table 4: Percentage distribution of APC ratio in the control subjects and as pregnancy progressed

APC ratio	Control (n/%)	2nd trimester (n/%)	3rd trimester (n/%)
≥4	42 (84)	107 (74)	56 (39)
<4	8 (16)	38 (26)	89 (61)
Total	50 (100)	145 (100)	145 (100)

X$^{[2]}$ value is $P<0.001$

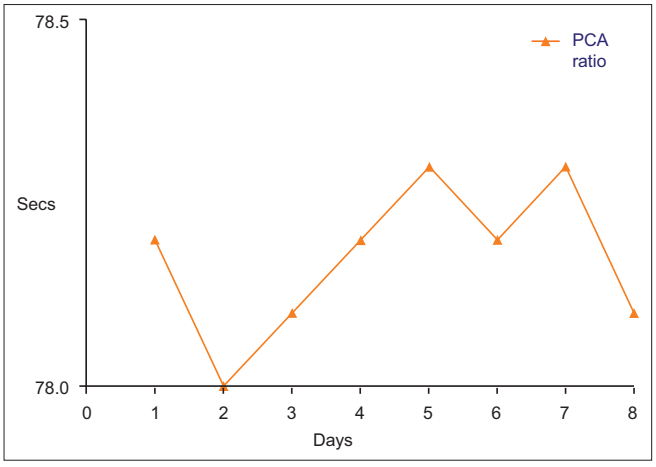

Figure 1: Trends in variation in the protein C activity among the abnormal reference plasma

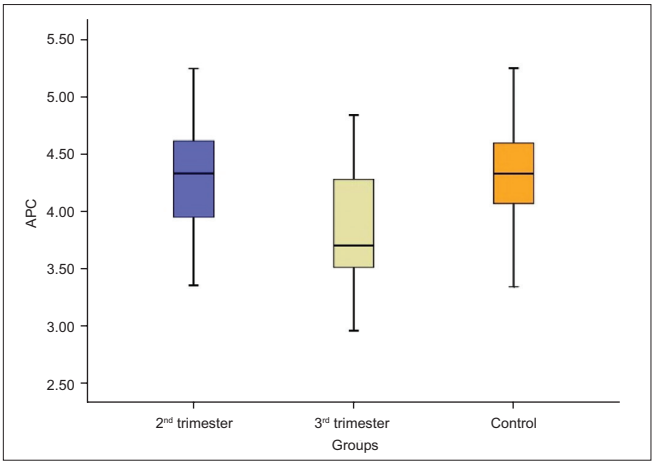

Figure 2: Trends in variation of PCA ratio among the pregnant and non pregnant subjects

The female non-pregnant controls and pregnant subjects in their 2nd and 3rd trimester with normal APC sensitivity ratio of ≥ 4 were 84%, 74%, and 39%, respectively. Women with APC sensitivity ratio < 4 were 16% for control 26% and 61% for 2nd and 3rd trimester, respectively. These differences were statistically significant ($P<0.0001$).

DISCUSSION

All the subjects were within the reproductive age group (16 - 48 years). Some of the subjects in the pregnant group were lost to follow-up. The main reason was their decision to get antenatal services in other hospitals nearer their areas of abode, after their first visit to the teaching hospital. Other reasons were voluntary decline from the study after the first sample collection, ill health, and changing of their geographical location.

The differences in age and parity present a selection bias as the study was conducted in a teaching hospital, and this resulted in most of the control subjects being college students. Expectedly, the weight and the BMI increased as pregnancy progressed. Though these women were recruited from the second semester, the amount of weight gained during a single pregnancy varies among women. It has been documented that the overall pregnancy weight gain for women starting pregnancy at a normal weight and with a BMI of 18.5 - 24.9, range from 11.4 to 15.9 kg.[34]

In this study, the mean APC sensitivity ratio (APTT with APC/APTT) reduced significantly as pregnancy progressed. The implication is an increase in protein C resistance. This was similar to reports where resistance to activated protein C increased in the 2nd and 3rd trimesters of pregnancy.[35-37] Indeed, a reduction of the ratio in the 1st and 2nd trimesters was associated with gestational complications, such as pregnancy loss, pre-eclampsia, and *abruptio placentae*.[35]

These changes can be attributed to increase in estrogen levels, increased plasma volume, increased levels of some procoagulant e.g. Factor VIII, decreased levels of some of the natural anticoagulants, and diminishing fibrinolysis.[7] The net effect is the establishment of a physiological, hyper-coagulable state, which is critical in achieving minimal blood loss during labor. Additionally, pregnancy-induced physiological changes reduce blood flow in the legs and lead to significant venostasis.[38] The combination of these factors may become potentially hazardous, with great potential for initiating thrombo-embolic events. This occurs more specifically when there are other associated risk factors, like cesarean delivery (especially emergency case, black race, obesity, multiple pregnancy, heart disease, diabetes, sickle cell disease, smoking, anti-phospholipid syndrome inherited thrombophilia, and previous history of thrombosis.[39-41]

In Nigeria, thromboembolic disorders have been reported as a rare cause of maternal mortality.[9-15] This is dangerously misleading and can obviously not be attributed to low prevalence rate. The real problems involve; inadequate study design, under diagnosis, and missed diagnosis due to diagnostic constraints and challenges. Others include non-reporting and under-reporting of cases. Indeed, aversion to autopsies due to the peculiar socio-cultural and economic situations prevalent in our environment tends to aggravate the situation.

This study is one of the first in Nigeria that utilized clot-(coagulometric) based assay to evaluate Protein C activity. Clot-based assays provide a more realistic account as it also tests the interaction of the APC and phospholipids, the PC cofactor protein S and the substrate factors Va and VIIIa, which are all necessary for APC anticoagulant activity *in vivo*. Furthermore, it highlights the presence of activated protein C resistance sometimes seen in pregnancy and in cooperates the *in vivo* interaction of factors involved in protein C system.[42] However, the study would have been more robust, if the women were recruited before pregnancy to assess the prevalence of inherited protein C resistance, then followed during pregnancy and childbirth. This will allow caregivers to determine the role of inherited and/or acquired protein C resistance on pregnancy outcome. Further studies will address these inadequacies, including the evaluation of factor VIII levels, which was not assayed in this study. Indeed, pregnant women with complicated pregnancies like hypertensive disorders and gestational diabetes mellitus should be assessed for thrombophilic conditions.

It is undisputable that hypercoagulability in pregnancy is not uncommon in our environment, and thromboembolic disorders may contribute as one of the factors militating against the reduction of maternal mortality and morbidity. As the deadline for MDGs fast approaches and deaths from PPH regresses,[19-21] as progress toward economic, manpower, and technological development are being made, it is appropriate that strategies and policy for the management of thromboembolic disorders be put in place. This will make our practitioners to perform proactively and prevent the devastating consequences of sudden death.

ACKNOWLEDGMENT

We acknowledge Dr. Emmanuel Nna of the Safety Molecular Pathology Laboratory UNEC Enugu for his contributions.

REFERENCES

1. Nathan DG, Orkin SH, Ginsburg D, Look TA. Hematology of infancy and childhood. 6th ed. In: Nathan and Oki, editors. Philadelphia: WB Saunders; 2003. p. 1631-67.
2. Butenas S, Mann KG. Blood coagulation. Biochemistry 2002;67:3-12.
3. Esmon CT, Esmon NL, Bonniec BF, Johnson AE. Protein C activation. Meth Enzymol 1993;222:359-85.
4. Esmon CT. The regulation of natural anticoagulant pathways. Science 1987;235:1348-52.
5. Hockin MF, Kalafatis M, Shatos M, Mann KG. Protein C activation and factor Va inactivation on human umbilical vein endothelial cells. Arterioscler Thromb Vasc Biol 1997;17:2765-75.
6. O'Riordan MN, Higgins JR. Haemostasis in normal and abnormal pregnancy. Best Pract Res Clin Obstet Gynaecol 2003;17:385-96.
7. Matsouka CJ. Haemostatic changes during pregnancy Haema 2005;8(Suppl 1):S68-71.
8. Faught W, Garner P, Jones J, Ivey B. Changes in protein C and protein S levels in normal pregnancy. Am J Obstet Gynecol 1995;172:147-50.
9. Onah HE, Okaro JM Umeh U, Chigbu CO. Maternal mortality in health institutions with emergency Obstetrics care Facilities in Enugu State Nigeria. J Obstet Gynaecol 2005;25:569-74.
10. Nwagha UI, Nwachukwu D, Dim V, Ibekwe PC, Onyebuchi A. Maternal mortality trend in South East Nigeria; less than a decade to the Millennium Developmental goals. J Womens Health 2010;19:323-7.
11. Aboyeji AP. Trends in maternal mortality in Ilorin. Trop J Obstet Gynaecol 1998;15:15-20.
12. Uja IA, Aisien OA, Mutihir JT, Vanderjagt DJ, Glaw RH, Uguru VE. Factors contributing to maternal mortality in North-Central Nigeria: A seventeen year review. Afr J Reprod Health 2005;9:27-40.
13. Audu LR, Ekele BA. A Ten year review of maternal mortality in Sokoto, Northern Nigeria. West Afr J Med 2002;21:74-6.
14. Uzoigwe SA, John CT. Maternal mortality in the University of Port Harcourt Teaching Hospital: PH in the last year before the New Millennium. Niger J Med 2004;13:32-5.
15. Oladapo TO, Lamina MA, Fakoya TA. Maternal deaths in Shagamu in the new millennium: A facility-based retrospective analysis. BMC Pregnancy Childbirth 2006;6:6.
16. Goswani A, Kasliwal MR, Lekharaj GH, Urala MS. Maternal mortality in a tertiary care centre in Nepal. Trop J Obstet Gynaecol 2004;21:168-71.
17. Oyieke IB. Millennium Development Goals 5: A review of maternal mortality at the Kenyatta National Hospital, Nairobi. East Afr Med J 2006;83:4-9.
18. Lema VM, Changole J, Kanyighe C, Malunga EV. Maternal mortality at the Queen Elizabeth Central Teaching Hospital, Blantyre, Malawi. East Afr Med J 2005;82:3-9.
19. Ajenifuja KO, Adepiti CA, Ogunniyi SO. Post partum haemorrhage in a teaching hospital in Nigeria: A 5-year experience. Afr Health Sci 2010;10:71-4.
20. Ijaiya MA, Aboyeji AP, Abubakar D. Analysis of 348 consecutive cases of Primary postpartum haemorrhage at a tertiary hospital in Nigeria. J Obstet Gynaecol 2003;23:374-7.
21. Kullima AA, Kawuwa MB, Audu BM, Geidam AD, Mairiga AG. Trends in maternal mortality in a tertiary institution in Northern Nigeria. Ann Afr Med 2009;8:221-4.
22. Hogan MC, ForemanKJ, Naghavi M, Ahn SY, Wang M, Makela SM, *et al*. Maternal mortality for 181 countries, 1980–2008: A systematic analysis of progress towards Millennium Development Goal 5. Lancet 2010;375:1609-23.
23. Adedirain IA, Durosinmi MA, Ogunniyi SO, Akinola NO, Akanmu AS. Haemostatic parameters in normal pregnant Nigerians and in Nigerians with hypertensive disorders of pregnancy. Niger Post Grad Med J 1999;6:49.
24. Akinsete I, Uyanwah PO. The fibrinolytic enzyme system in pregnancy in Nigerians. Afr J Med Med Sci 1989;18:89-93.
25. Obisesan KA, Adeyemo AA, Okunade MA. Haematological values in pregnancy in Ibadan, Nigeria. Afr J Med Med Sci 1998;27:9-11.
26. Onwukeme KE, Uguru VE. Haematological values in pregnancy in Jos. West Afr J Med 1990;9:70-5.
27. Akingbola TS, Adewole IF, Adesina OA, Afolabi KA, Fehintola FA, Bamgboye EA, *et al*. Haematological profile of healthy pregnant women in Ibadan, south-western Nigeria. J Obstet Gynaecol 2006;26:763-9.
28. State Ministry of Health, Enugu Health Sector Reform: Implementing the District Health System. Posted 2004. Available from: http://www.enugustate.gov.ng/. [Last assessed on 2010 Aug 30].
29. Ezugwu EC, Onah HE, Ezugwu FO, Okafor II. Maternal Mortality in a Transitional Hospital in Enugu, South East Nigeria. Afr J Reprod Health 2009;13:67-72.
30. Campbell MJ, Machin D. Medical Statistics; a common sense Approach New Jersy: John Wiley and sons; 1996. p. 156-7.
31. Okunola MA, Avinde OA, Owonikoko KM, Omigbodun AO. Factors influencing gestational Age at antenatal booking at the University College Hospital, Ibadan Nigeria. J Obstet Gynaecol 2006;26:195-7.
32. Nwagha UI, Ugwu OV, Nwagha TU, Anyaehie USB. The influence of parity on the gestational age at booking among pregnant women in Enugu, South East Nigeria. Niger J Physiol Sci 2008;23:67-70.
33. Idowu OA, Mafiana CF, Sotiloye D. Anaemia in pregnancy: A survey of pregnant women in Abeokuta, Nigeria. Afr Health Sci 2000;5:295-9.
34. Broughton-Pipkin F. Maternal Physiology. Dewhurst Textbook of Obstetrics and Gynaecology. 7th ed. In: Edmonds DK, editor. Oxford: Blackwell Publishing; 2007. p. 10-18.
35. Mathonnet F, de Mazancourt P, Bastenaire B, Morot M. Activated protein C sensitivity ratio in pregnant women at delivery. Br J Haematol 1996;92:244-6.
36. Walker C, Garner R, Keely EJ, Rock GA, Reis MD. Changes in activated protein C resistance during normal pregnancy. Am J Obstet Gynecol 1997;177:162-9.
37. Cumming AM, Tait RC, Fildes S, Yoong A, Keeney S, Hay CR. Development of resistance to activated Protein c during pregnancy. Br J Haematol 1995;90:725-7.
38. Macklon NS, Greer IA, Bowman AW. An ultrasound study of gestational and postural changes in the deep venous system of the leg in pregnancy. Br J Obstet Gynaecol 1997;104:191-7.
39. Knight M, UKOSS. Antenatal pulmonary embolism: Risk factors, management and outcomes. BJOG 2008;115:453-61.
40. Larsen TB, Sørensen HT, Gislum M, Johnsen SP. Maternal smoking, obesity, and risk of venous thromboembolism during pregnancy and the puerperium: A population-based nested case-control study. Thromb Res 2007;120:505-9.
41. James AH, Jamison MG, Brancazio LR, Myers MR. Venous thromboembolism during pregnancy and the postpartum period: Incidence, risk factors, and mortality. Am J Obstet Gynecol 2006;194:1311-5.
42. Marlar RA, Adock DM. Clinical evaluation of Protein c: A comparative review of antigenic and functional assay. Hum Pathol 1989; 20:1040-7.

How to cite this article: Nwagha UT, Nwagha UI, Ibegbulam OG, Ocheni S, Okpala I, Ezeonu PO, *et al*. Increased prevalence of activated protein C resistance during pregnancy may implicate venous thrombo embolic disorders as a common cause of maternal mortality in Nigeria. J Basic Clin Reprod Sci 2012;1:19-24.

Source of Support: Nil, **Conflict of Interest:** None declared

ISSN - 0000-0000

JOURNAL OF BASIC and CLINICAL REPRODUCTIVE SCIENCES

Official Publication of Society of Reproductive Biologist of Nigeria

Volume 1 / Issue 1 / Year 2012

www.jbcrs.org