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# The final stage: Investigating determinants of plasmin generation in the third trimester of pregnancy

**H C Okoye**,[1] MBBS, MSc, MD, FMCPath, FWACP; **T U Nwagha**,[1] MBBS, MSc, MD, FMCPath; **J T Enebe**,[2] MBBS, MSc, FMCOG; **C Efobi**,[3] MBBS, PhD, FMCPath; **O C Nnachi**,[4] MBBS, FMCPath, FWACP; **C J Okwor**,[5] MBBS, FMCPath

[1]*Department of Haematology and Immunology, College of Medicine, University of Nigeria Ituku-Ozalla, Enugu, Nigeria*
[2]*Department of Obstetrics and Gynaecology, College of Medicine, Enugu State University of Science and Technology Teaching Hospital Parklane, Enugu, Nigeria*
[3]*Department of Haematology and Blood Transfusion, College of Health Sciences, Nnamdi Azikiwe University Nnewi, Nnewi, Nigeria*
[4]*Department of Haematology and Blood Transfusion, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria*
[5]*Department of Chemical Pathology, College of Medicine, University of Nigeria Ituku-Ozalla, Enugu, Nigeria*

***Corresponding author:*** *T U Nwagha (theresa.nwagha@unn.edu.ng)*

**Background.** Changes in the fibrinolytic system that support haemostasis are seen in the third trimester of pregnancy. An imbalance between coagulation and fibrinolysis could increase either thrombotic episodes or haemorrhagic risk.
**Objective.** To assess plasma D-dimer and plasmin-a2-antiplasmin (PAP) complex levels as indication of plasmin generation during late pregnancy.
**Methods.** A sample of 41 healthy pregnant women in their third trimester participated in the study. Data on obstetric history, body mass index (BMI) and other demographic variables were recorded. Haematological analysis involved enzyme-linked immunosorbent assaying of venous blood samples. Statistical analysis was performed using SPSS version 21.
**Results.** Mean maternal and gestational age, with associated standard deviations (SD), were 30.68 (4.69) years and 34.78 (3.34) weeks, respectively. Haematological analysis showed mean (SD) values of D-dimer and PAP complex to be 194 (24 ng/mL) and 175 (11 ng/mL), respectively. Both indicators were positively correlated with maternal age, gestational age and BMI grouping, although significantly only for maternal age. Multiple linear regression analysis showed that with every increasing year of maternal age, D-dimer levels increased by 2.0 ng/mL (95% confidence interval (CI) 0.4 - 3.6 ng/mL). PAP complex levels similarly increased by 0.8 ng/mL (95% CI 0.1 - 1.5 ng/mL), after controlling for gestational age and BMI.
**Conclusion.** D-dimer and PAP levels increased with increasing maternal age, suggesting it to be an independent determinant of plasmin generation in the third trimester of pregnancy. If this finding is confirmed in larger studies, age should be considered when interpreting these indicator values during pregnancy.
**Keywords**. XX

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Normal pregnancy is a hypercoagulable state during which procoagulation is promoted and fibrinolysis is limited.[1,2] This encourages thrombosis and reduces haemorrhagic risk, particularly during the third trimester. There is increased expression and upregulation of coagulant factors, fibrinogen and antifibrinolytic proteins[3] to prepare the body for the acute and extreme haemostatic challenge resulting from the separation of the placenta.[4] Rapid and effective control of haemostasis of the placental bed improves both maternal and fetal outcomes in a normal pregnancy.

Thrombin generation and breakdown of fibrinogen characterise the tilt towards procoagulation seen in late pregnancy. Fibrin so produced also encourages fibrinolysis and promotes plasmin generation.[5] The plasmin-$\alpha_2$-antiplasmin (PAP) complex has an important role in blood coagulation and fibrinolysis. It controls and regulates clot dissolution into soluble fragments and is an index of recent fibrinolytic activity.[6] Its role in counterbalancing the coagulation process in the third trimester ensures effective haemostatic control during the third stage of labour. Even though fibrinolysis is thought to be reduced in pregnancy, there is evidence of active fibrinolysis as pregnancy progresses. D-dimer, a product of fibrin degradation, increases as a pregnancy progresses[7] and is indicative of active fibrinolysis.

The loss of fibrinolytic activity is presumed to be due to the loss of plasminogen activators, as shown by a normal fibrinolytic response when these proteases are added in excess to a urokinase sensitivity test. However, the capacity for localised fibrinolytic activity is not lost, as slightly raised levels of fibrinolytic degradation products are noticed during pregnancy.

An imbalance between coagulation and fibrinolysis during pregnancy could increase either thrombotic episodes or haemorrhagic risk, especially in late pregnancy. Whereas coagulation in normal pregnancy has been extensively studied,[8,9] there is a need to better understand the fibrinolytic process and its controls.

In this study, we measured PAP and D-dimer levels and their determinants in a sample of women with normal pregnancies presenting at a tertiary health facility in Nigeria.

# Methods

## Study design and setting

This was a cross-sectional study carried out at the University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu, a federal tertiary healthcare institution that serves as a referral centre mainly for Enugu State. Few referrals come from neighbouring states.

## Study participants and sample size

All women in the third trimester of a normal pregnancy and attending a routine antenatal visit were included in the study. Women with comorbidities, twin gestation, evidence of intrauterine growth restriction or other obstetric complications were excluded.

Gestational age was confirmed either by an early obstetrics ultrasound scan or based on the last menstrual cycle (for those that had a normal 28-day cycle). Participants' obstetrics history, their weight (kg), height (m) and body mass index (BMI) were recorded.

To determine the required sample size, we used Cochran's formula with a *Z*-score of 1.96, and assuming 12% as the rate of pregnant women with normal limits of D-dimer,[10] set the acceptable error limit at 10% and assumed a 10% non-response rate. The calculation showed a sample size of 40 participants was required.

## Haematological analysis

Blood samples (4 mL) were drawn into ethylene diamine tetraacetic acid vials from each subject and processed within an hour of collection by centrifuging at 1500 *g* for 10 minutes. Aliquots of platelet-poor plasma were separated, labelled and stored in plastic tubes at –80 °C until analysis. Stored samples were thawed at room temperature for analysis and plasma levels of D-dimer and PAP were determined using ELISA kits (Elabscience®, USA) according to the manufacturer's protocol.

## Statistical analysis

Data were entered into a spreadsheet and then exported to SPSS (version 21) for statistical analysis. Means, standard deviation, median and ranges were used to describe numerical variables whereas frequencies and percentages were used to describe categorical variables. Student's *t*-test and one-way analysis of variance were used to compare mean PAP complex and D-dimer levels across groups. Correlation and multiple linear regression analyses were used to explore the relationship between demographic variables (maternal age, gestational age and BMI) and dependent variables (PAP complex and D-dimer levels). Statistical significance was set at $p<0.05$.

## Ethical considerations

Approval for this study was obtained from the Health Research Ethics Committee of the University of Nigeria Teaching Hospital (ref. no. NHREC/05/01/2008B-FWA00002458-1RB00002323). The objective of the study, together with the risks and benefits, were explained to the participants in the language they understood. Only participants who consented to participation and met the inclusion criteria were included in the study.

# Results

A total of 41 women participated in the study. Ages ranged between 19 and 39 years, with two-thirds (65.9%) of the women being between 30 and 39 years old (Table 1). The mean (standard deviation, SD) maternal age was 30.68 (4.69) years. Mean (SD) gestational age among the sample was 34.78 (3.34) weeks). Mean (SD) BMI was 28.84 (4.29) kg/m$^2$. BMIs were classified as normal (18.5 - 24.9), overweight (25.0 - 29.9) or obese (≥30). Only seven (17.1%) of the participants had a normal BMI (Fig. 1).

The mean (SD) D-dimer level was 194 (24) ng/mL, within a range of 168 - 276 ng/mL (median: 184 ng/mL). The mean (SD) PAP complex level was 175 (11) ng/mL, within a range of 156 - 200 ng/mL (median: 172 ng/mL).

Levels of both D-dimer and PAP complex were positively correlated with maternal age, gestational age and BMI grouping, but statistically significant only for maternal age (Table 2).

Results from multiple linear regression analysis (Supplementary Table 1) showed that with every unit increase in maternal age, D-dimer levels increased by 2.0 ng/mL ($p=0.031$; 95% confidence interval (CI) 0.4 - 3.6 ng/mL). Similarly, PAP complex levels increased by 0.8 ng/mL ($p=0.016$; 95% CI 0.1 - 1.5 ng/mL) for every unit increase in maternal age (Supplementary Table 2).

Mean D-dimer and PAP complex levels increased across maternal age categories, but the differences were not statistically significant. There was no significant difference in D-dimer or PAP complex levels across the different gestational age or BMI categories (see Table 3).

# Discussion

The physiological changes in pregnancy favour procoagulation, which reduces the risk of haemorrhage following placental separation during childbirth. There is evidence that active fibrinolysis and plasmin generation occur in the later stages of pregnancy[5] to achieve haemostatic balance. This study evaluated some markers of active fibrinolysis and plasmin generation in the third trimester of normal pregnancy.

In our sample, D-dimer and PAP complex levels were within the physiological reference ranges. The mean serum levels of these markers of active fibrinolysis were similar to those reported by Simioni and Campello,[11] who showed that fibrinolytic activity rises together with increased activation of coagulation during normal pregnancy.

However, the levels of D-dimer seen in our study were lower than what was reported in a study among Polish women.[12] This suggests

**Table 1. Maternal and gestational age of study participants (*N*=41)**

| Variables | *n* (%) |
|---|---|
| Maternal age (years) | |
| <20 | 1 (2.4) |
| 20 - 29 | 13 (31.7) |
| 30 - 39 | 27 (65.9) |
| Gestational age (weeks) | |
| 26 - 30 | 5 (12.2) |
| 31 - 35 | 15 (36.6) |
| 36 - 40 | 21 (51.2) |

**Table 2. Correlations (Pearson) between levels of fibrinolytic indicators (D-dimer and PAP complex) and maternal age, gestational age and BMI group**

| Variables | D-dimer, *r* (*p*-value) | PAP, *r* (*p*-value) |
|---|---|---|
| Maternal age | 0.336 (0.032)* | 0.373 (0.016)* |
| Gestational age | 0.131 (0.413) | 0.189 (0.238) |
| BMI | 0.070 (0.663) | 0.137 (0.394) |

*Statistically significant ($p<0.05$)
PAP = plasmin-$\alpha_2$-antiplasmin; BMI = body mass index

**Table 3. Comparison of mean D-dimer and PAP complex levels by maternal age, gestational age and BMI classification (N=41)**

| Variables | D-dimer (ng/mL), mean (SD) | F (*p*-value) | PAP complex (ng/mL), mean (SD) | F (*p*-value) |
|---|---|---|---|---|
| Maternal age (years) | | | | |
| <20 | 178 (5) | | 164 (4) | |
| 20 - 29 | 185 (13) | 1.750 (0.187) | 170 (6) | 2.569 (0.090) |
| 30 - 39 | 199 (27) | | 177 (12) | |
| Gestational age (weeks) | | | | |
| 26 - 30 | 192 (26) | | 178 (13) | |
| 31 - 35 | 190 (19) | 0.368 (0.694) | 175 (11) | 0.458 (0.636) |
| 36 - 40 | 197 (27) | | 174 (10) | |
| BMI classification (kg/m2) | | | | |
| Normal (18.5 - 24.9) | 179 (20) | | 181 (12) | |
| Overweight (25.0 -29.9) | 198 (19) | 1.604 (0.214) | 174 (9) | 1.262 (0.295) |
| Obese (≥30.0) | 195 (29) | | 173 (11) | |

PAP = plasmin-$\alpha_2$-antiplasmin; BMI = body mass index; SD = standard deviation

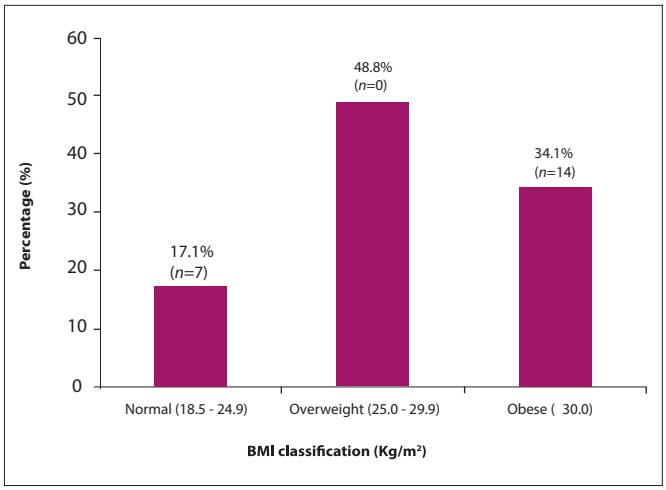


*Fig. 1. Distribution of participants according to BMI grouping*
*BMI = body mass index.*

that D-dimer levels during pregnancy should be interpreted relative to appropriate reference ranges. Although the reason for the lower levels of D-dimer reported in our study could not be confirmed, racial differences may be possible; further investigation would be required.

Lower plasma levels of D-dimer at a gestational age of 31 - 35 weeks, with levels appearing to pick up at 36 - 40 weeks, might be attributed to the circadian rhythm of some plasma proteins, but could not be confirmed from the results in our study. A similar pattern was not reported by Siennicka *et al.*,[12] who found a global rise of D-dimer levels in the third trimester; the period was not subcategorised in our study. The small sample size used in our study may also have contributed to this .

The levels of PAP complex were similar to those reported by Uszynski *et al.*,[13] whose study focused on women in the third trimester of pregnancy through to parturition. Their results were in line with the expected physiologic changes in indicators of fibrinolysis in the third trimester in order to reduce haemorrhagic risk and low levels of PAP complex were found in umbilical fluid. The level of PAP complex in umbilical fluid was not assessed in our study.

Owing to the physiological changes seen in the haemostatic system, interpreting the levels of fibrinolytic markers according to reference ranges for pregnant women may be ambiguous, as they could be affected by demographic and obstetric factors such as the mother's age, gestational age and BMI. In our study, maternal age was found to be an independent determinant of plasmin generation in the third trimester, and levels of D-dimer and PAP complex increased with increasing maternal age. If this finding is confirmed in larger studies, maternal age should be considered when interpreting D-dimer and PAP values during pregnancy.

The findings of this study contribute to current understanding of the fibrinolytic process and its controls during pregnancy in that they provide a snapshot of serum levels of D-dimer and PAP complex in the third trimester.

## Study limitations

A longitudinal (instead of cross-sectional) design could have made the study more robust, especially if women were followed until parturition. Comparing indicator levels with a non-pregnant group could also have contributed to strength of interpretation.

# Conclusion

This study highlights variations in D-dimer and PAP complex levels in late pregnancy, emphasising the need for population-specific reference ranges and individualised assessments. Maternal age influences fibrinolysis, and further research into circadian rhythms and demographic factors could refine clinical guidelines for maternal health.

**Declaration**

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**Author contributions.** HO and TN conceptualised and designed the study, with additional input from JE, CE, ON and CO. All authors contributed to data screening and reviewed earlier drafts of the manuscript and approved the final version.

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