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Assessment of Fibrinolytic Activities Among Pregnant Women in Etsako West, Edo State

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Abstract

Pregnancy alters haemostasis throughout the trimesters, impacting both mother's and baby's health, particularly during complications. This study investigated fibrinolytic profiles (tissue plasminogen activator [tPA] and clot lysis time) among primigravidae and multigravidae in Etsako West Local Government area of Edo State, Nigeria. The aim was to emphasize the importance of timely and accurate diagnosis of clotting disorders in expectant mothers. This study was a cross-sectional study of primigravidae and multigravidae. A random sampling technique was used to recruit two hundred women attending antenatal clinics in the selected hospitals. Euglobulin clot lysis time (ECLT) and tissue plasminogen activator (tPA) were measured. Immunoturbidimetric and chromogenic techniques were used to assess tPA levels. Inferential statistics were used to test for association between categorical variables and statistical significance set at p -value < 0.05 . Significant differences ($p < 0.001$) were observed for both tPA and ECLT across all trimesters in primigravidae. Gravidity significantly impacted tPA levels and ECLT ($P < 0.001$). Age did not affect fibrinolytic activities of pregnant women. Haemostatic protein should be monitored during pregnancy to assess complication risks like consumptive coagulopathy, while tPA offers valuable insights into pregnancy health and prognosis.

Key words: Fibrinolysis, primigravidae, multigravidae, haemostasis, complications.

Introduction

Pregnancy is a remarkable physiological transformation that significantly alters a woman's body to nurture a growing foetus. This includes

intricate modifications within the blood clotting system, or haemostasis, to ensure adequate blood flow to the developing foetus while maintaining the mother's health. However, maintaining this delicate balance between clot formation and breakdown, known as fibrinolysis, becomes crucial throughout pregnancy, especially during complications like preeclampsia or placental abruption (Oladosu-Olayiwola *et al.*, 2018). At the heart of this balance lies plasmin, a powerful enzyme responsible for dissolving blood clots. Plasmin itself is produced in an inactive form called plasminogen by the liver. Although inactive, plasminogen exhibits a unique affinity for fibrin, the main protein component of blood clots. This allows plasminogen to become incorporated into the clot structure as it forms, strategically positioning it for action once activated (Oladosu-Olayiwola *et al.*, 2018). The activation of plasminogen into its active form, plasmin, is orchestrated by specific enzymes called plasminogen activators (Longstaff and Kolev, 2015). Tissue plasminogen activator (tPA) is a key player released by the damaged lining (endothelium) of blood vessels. This controlled release ensures that clot breakdown occurs gradually, typically after several days after bleeding has stopped (Geoffrey, 2005). This delayed activation is crucial to prevent excessive bleeding during the initial stages of clot formation. Notably, plasminogen becomes trapped within the clot itself, allowing it to be efficiently broken down by the activated plasmin as it slowly works its way through the fibrin mesh (Cesarman and Hajer, 2018).

However, the activation process is regulated to prevent uncontrolled breakdown of clots. Plasminogen activator inhibitors (PAIs), particularly PAI-1 and PAI-2, act as natural brakes

on the plasminogen activator system, effectively controlling plasmin generation (Cesarman and Hajer, 2018). Interestingly, plasmin itself participates in a feedback loop, stimulating the production of more active forms of tPA and urokinase (another plasminogen activator), further promoting clot breakdown (Cotran *et al.*, 2009).

On the other hand, other mechanisms exist to inactivate plasmin directly. Alpha-2 antiplasmin and alpha-2 macroglobulin are protease inhibitors that effectively neutralize plasmin activity. Additionally, thrombin-activatable fibrinolysis inhibitor (TAFI) modifies fibrin within the clot, making it more resistant to plasmin-mediated degradation (Cesarman and Hajer, 2018). This intricate interplay between plasminogen activation, inhibition, and plasmin inactivation ensures a finely tuned fibrinolytic system throughout the body. Understanding the dynamics of fibrinolysis during pregnancy is particularly important. Pregnancy itself induces a hypercoagulable state, meaning an increased tendency for blood clotting. This is a necessary adaptation to prevent excessive bleeding during childbirth. However, it also raises the risk of complications arising from blood clots (Cesarman and Hajer, 2018).

Previous research has explored the alterations in the coagulation cascade during pregnancy, although, limited data exists on how the fibrinolytic system specifically adapts and functions throughout pregnancy, particularly in primigravidae compared to multigravidae (Lynch *et al.*, 2019). Primigravidae embark on a unique journey with their first pregnancy, and successful management of this initial experience can generally impact their subsequent obstetrical outcomes (Lynch *et al.*, 2019). Investigating potential differences in the fibrinolytic profile between primigravidae and multigravidae in Etsako West, Edo State, could provide valuable insights into how pregnancy adaptations and prior obstetric experiences might influence this critical physiological system.

The aim of this is to address a gap in knowledge by investigating the fibrinolytic profiles of primigravidae and multigravida women in Etsako, Edo North, Nigeria. The study sought to identify potential differences in fibrinolytic

activity between these two groups. This knowledge could contribute to the development of improved diagnostic and therapeutic strategies for pregnancy-related complications associated with abnormal clot breakdown.

Methodology
Study Design.

The study was a cross-sectional study where blood samples of primigravidae and multigravidae in Etsako West Local Government Area, Edo State were analyzed for fibrinolytic profiling. The cost and expenses incurred during this research were borne by the researchers.

Sample Size Determination

The sample size was determined by applying Fisher (1998)'s formula as follows:

$$\text{Samples size (N)} = \frac{Z^2 Pq}{d^2}$$

Where.

N = the desired size

Z = 1.96 (standard score)

P = prevalence (12%) (0.12) (Oluwaseyi *et al.*, 2021)

Q = 1 - P (1 - 0.12 = 0.88)

D = sample error tolerated (0.05)

$$N = \frac{1.96^2 \times 0.12 \times 0.88}{0.05^2}$$

$$= 162.3 \text{ approximately} = 162$$

A non-response rate of 10% of 162 = 16.2 approximately 16

$$N = 162 + 16 = 178$$

Sample size was rounded up to 200, to take care of attrition.

Study Population and Subjects

The study recruited primigravidae and multigravidae attending antenatal clinics at three hospitals in Edo State, Nigeria: General Hospital Agbode (n=50), Edo State University Teaching Hospital Auchi (n=100), and General Hospital Jagbe (n=50). A quasi-random sampling of selecting every fifth pregnant woman in the facility was adopted until the sample size was met. Participants were aged 18 to 45 years old.

Sampling Techniques

Two hundred pregnant women were recruited using a quasi-random sampling method from antenatal clinics at selected hospitals in Etsako

West, Nigeria. These women served as participants for data collection and blood sampling. Questionnaires were used to collect the clinical history and gestational age of subjects. Blood samples were collected from participants across all three trimesters.

Ethical Considerations

This study was approved by the Edo State University Teaching Hospital, Uzairue Research and Ethics Committee with approval number (EDSUREC24/0001) and written informed consent was obtained from all participants. Study participants were assured of strict confidentiality. Participation was voluntary and participants were offered the choice to pull out at any point of the study.

Data Analysis

The data generated from this study was statistically analyzed using the International Business Machines Corporation (IBM) SPSS version 29 to determine the mean, standard deviation at 0.05 level of significance and 95% confidence interval.

Sample Preparation

A volume of 4.5ml of blood was added to a tube containing sodium citrate in a 1:9 ratio (blood to citrate) and mixed thoroughly. This sample was then centrifuged at high speed to isolate platelet-poor plasma. The plasma was dispensed into two cryovials and stored at -20°C for analysis.

ELISA for the analysis of Tissue plasminogen activator

This research employed an enzyme-linked immunosorbent assay (ELISA) kit to quantify human tissue plasminogen activator (t-PA) levels in samples.

Plate Coating: The wells of a microtiter plate were coated with a purified human t-PA antibody, creating a solid phase for capturing t-PA antigens.

Sample and Conjugate Addition: The prepared samples containing t-PA were then added to the wells, allowing them to bind to the immobilized antibody. Subsequently, a horseradish peroxidase (HRP)-labeled anti-t-PA antibody was introduced. This created an "antibody-antigen-enzyme-antigen" complex.

Washing and Substrate Incubation: Unbound materials were removed through a thorough washing step. Next, a tetramethylbenzidine (TMB) substrate solution was added. HRP within the formed complexes catalyzed the conversion of TMB to a blue-colored product.

Colorimetric Detection and Analysis: The enzyme reaction was stopped by adding a sulfuric acid solution, and the resulting colour change was measured at a wavelength of 450 nm. The optical density (OD) values of the samples were then compared to a standard curve generated with known concentrations of human t-PA. This comparison allowed for the determination of the t-PA concentration within the tested samples.

Euglobulin Clot Lysis Time Test (ECL-T)

The research also employed the Euglobulin Clot Lysis Time (ECL-T) assay to assess overall fibrinolytic activity in the blood samples. This procedure involved the following steps:

Blood Collection and Plasma Preparation: Blood samples were collected from participants and centrifuged at low speed. The resulting platelet-poor plasma was then separated and used for further processing.

Acidification and Euglobulin Precipitation: An acidic solution was added to the plasma, causing precipitation of certain proteins including fibrinogen, plasminogen, and factors involved in clot formation and breakdown. This precipitate, known as the euglobulin fraction, was harvested.

Recalcification and Clot Formation: The euglobulin fraction was resuspended in a buffer solution containing calcium chloride. This triggered the formation of a fibrin clot within the solution.

Clot Lysis Monitoring: The test tubes containing the formed clots were incubated at a specific temperature (typically 37°C). The time it took the clot to completely lyse (dissolve) visually at regular intervals.

Interpretation: The time it took for complete clot lysis was recorded as the Euglobulin Clot Lysis Time (ECLT). Shorter ECLT values indicated increased fibrinolytic activity, while longer times suggested reduced clot breakdown potential. A positive test is evident with a shortened ELT (usually < 60 minutes).

Results

Socio demographic Features of the Participants

The mean age in years for the 200 subjects was 28.88±4.99 years. The majority of respondents were between the ages of 24-28 years. In addition, more than half of the participants 111(55.5%) were multigravida and 128 (64.0%) were in their second

trimester. The majority had a secondary level of education 129 (64.5%) while the minority had a tertiary level of Education 4 (2%). The majority of the participants have had one pregnancy 90(45%) while the minority have had five pregnancies 2(1.0%), Majority of the participants were from Etsako 169(84.5) while the minority of the participants were Fulani 1(0.5).

Table 1: Sociodemographic Characteristics of Study Participants

Variable	Frequency (N=200)	Percentage
Age (Mean age: 28.88±4.99 Years)		
19-23	33	16.5
24-28	61	30.5
29-33	56	28.0
34-38	49	24.5
39-43	1	0.5
Status of Pregnancy		
Primigravida	89	44.5
Multigravida	111	55.5
Gestational Age		
First Trimester	6	3.0
Second Trimester	128	64.0
Third Trimester	66	33.0
Educational Level		
Primary Education	67	33.5
Secondary Education	129	64.5
Tertiary Education	4	2.0
Gravidity		
1	113	56.5
2	69	34.5
3	9	4.5
4	7	3.5
5	2	1.0
Ethnicity		
Etsako	169	84.5
Esan	7	3.5
Yoruba	9	4.5
Hausa	5	2.5
Bini	4	2.0
Akoko Edo	1	0.5
Igbo	4	2.0
Fulani	1	0.5
Awareness of Antenatal Testing		
Aware	200	100
Awareness of Coagulation Disorders		
Aware	1	0.4
Unaware	199	99.6
Participants Attitude to Testing		
Strongly Recommended	200	100
Have you been diagnosed with coagulation disorder?		
No	200	100

*The frequency for age groups don't add up. The total is 203 instead of 200. Please correct

Knowledge and awareness

All the participants were aware of antenatal testing, however the majority 199(99.6%) were unaware of various coagulation disorders while minority 1(0.4%) were aware. None of the participants affirmed to have been diagnosed with coagulation disorders of any type. The participants attitudes towards participation were strongly positive.

Association between the gestational age and the mean of the parameters (t-PA and ECL-T)

Two hundred (200) women participated in this study. The study investigated the changes in fibrinolysis-related biomarkers throughout pregnancy and assessed potential differences between trimesters. First trimester, early stage of pregnancy (up to 13 weeks), second trimester, middle stage of pregnancy (14 to 26 weeks). Third trimester, late stage of pregnancy (27 weeks to

delivery). Table 1 presents data on two parameters, t-PA (tissue plasminogen activator) levels and Euglobulin Clot Lysis Time (ECL-T), across different trimesters of pregnancy. The data shows a significant variation in t-PA levels across trimesters. Specifically, t-PA levels are highest in the second trimester (22.99 ng/l), followed by the first trimester (22.12 ng/l), and lowest in the third trimester (20.82 ng/l). The observed decrease in t-PA levels towards the third trimester suggests potential regulation of fibrinolysis as pregnancy progresses. The t-PA levels showed a statistically significant difference across trimesters (P < 0.001), and there is a significant difference in Euglobulin Clot Lysis Time among trimesters. The longest clot lysis time is observed in the first trimester (158.0 mins), followed by the second trimester (152.95 mins), and the shortest in the third trimester (144.27 mins). ECL-T values exhibited significant variation among trimesters (P<0.001).

Table 1: Association between the gestational age and the mean of the parameters

Parameters	1 st Trimester N=200	2 nd Trimester N=200	3 rd Trimester N=200	P-value
t-PA (ng/l)	22.12±4.43	22.99±15.95	20.82±2.83	<0.001
ECL-T (Mins)	158.0±28.39	152.95±41.62	144.27±42.07	<0.001

Association between Gravidity and the mean of the Parameters

The data comparing two parameters, t-PA (tissue plasminogen activator) levels and Euglobulin Clot Lysis Time (ECL-T), between primigravidae (women pregnant for the first time) and multigravidae (women who have been pregnant more than once) groups is shown in Table 2. The study investigated potential differences in these parameters based on gravidity status.

The data shows that t-PA levels are slightly higher in primigravidae compared to multigravidae women (21.57ng/l and 21.11ng/l). The P-value (p<0.001) indicates a statistically significant difference between these groups, suggesting that t-PA levels vary based on gravidity status. Similarly, the Euglobulin Clot Lysis Time is longer (indicating slower clot lysis) in primigravida compared to multigravida women (151.39 mins and 149.31 mins).

Table 2: Association Between Gravidity and the Mean of the Parameters

Parameters	Primigravida (N=89)	Multigravida(N=111)	P=Value
t-PA (ng/l)	21.57±3.19	21.11±3.34	<0.001
ECL-T (mins)	151.39±38.86	149.31±43.66	<0.001

Association Between Participants' Age and the Parameters

Women who participated in this study were between the ages of 19-43. Two variables, t-PA (tissue plasminogen activator) and euglobulin clot lysis time were presented across the different age groups. The t-PA levels appear relatively consistent across the age groups, with minor

fluctuations within the mean values. In contrast, the E-CLT shows more variation across age groups. Notably, there is a decrease in clot lysis time from younger to older age groups, with the shortest time observed in the 29-33 years age group. The P-value ($p < 0.010$) suggests a statistically significant difference in clot lysis time among the age groups (Table 3).

Table 3: Association Between Participants' Age and the Parameters

Variables	19-23(years)	24-28(years)	29-33(years)	34-38(years)	39-43(years)	P-Value
t-PA	21.01±3.7	21.89±3.2	21.27±3.5	21.02±2.	21.93	>0.49
ECL-T	0	7	4	58	156.0	2
	165.88±4	145.16±3.	138.02±30	159.86±4	156.0	<0.01
	6.47	27	.12	5.35		0

Discussion

This study observed significant associations between gestational age and key fibrinolytic parameters like tissue plasminogen activator (tPA) and Euglobulin clot lysis time (ECLT) across all trimesters. The tPA is a crucial enzyme in fibrinolysis, maintaining a balance between blood clotting and breakdown. The observed fluctuations in tPA levels throughout pregnancy (increasing slightly in the second trimester and then decreasing in the third) point towards dynamic changes in fibrinolytic activity. This complex interplay between fibrinolysis and coagulation during gestation has been reported in previous research. A previous report documented similar trends in tPA levels across trimesters, supporting the concept of physiological alterations in fibrinolytic activity (Kam and Lee, 2018).

ECLT is another important marker of fibrinolytic activity. The observed decrease in ECLT during the second trimester followed by a slight increase in the third trimester suggests variations in fibrinolytic potential throughout pregnancy. This aligns with previous research by Brenner, who demonstrated changes in ECLT across trimesters, highlighting the dynamic regulation of fibrinolysis during gestation (Brenner *et al.*, 2014).

A significant association was found between gravidity and the fibrinolytic parameters (tPA

and ECLT). Mean tPA levels were slightly higher in primigravidae compared to multigravidae. This suggests potential differences in fibrinolytic activity between these groups. While this difference may not be clinically significant, it reflects variations in haemostatic profiles based on gravidity status. Research on tPA levels in primigravidae and multigravidae is limited, but previous studies exploring the link between fibrinolytic activity and pregnancy highlight the need for further investigation in this area (Ranta *et al.*, 2001; Fujimoto *et al.*, 2015).

Primigravidae also exhibited slightly higher mean ECLT compared to multigravidae. This suggests a potential difference in fibrinolytic potential between the two groups, with primigravidae possibly having a slower fibrinolytic response. While research directly comparing ECLT in primigravidae and multigravidae is limited, studies by Brenner and Santillan reported alterations in fibrinolytic activity during pregnancy, emphasizing the need for further exploration in this aspect (Brenner *et al.*, 2014; Santillan *et al.*, 2016).

No significant association was found between age and tPA concentration or ECLT. This suggests that age does not have a major impact on these fibrinolytic parameters in this study population. Research on the relationship between age and tPA has yielded mixed results. While Thompson observed age-related increases

in tPA levels in older adults (Thompson *et al.*, 2003), Juhan-Vague *et al.* (1996) did not find a significant association. These discrepancies might be due to differences in populations and study settings. Similarly, the lack of association between age and ECLT in this study aligns with some previous research, such as the study by Tofler which reported no significant age-related changes in ECLT (Tofler *et al.*, 1992). Further investigation is needed to clarify the relationship between age and fibrinolytic activity.

Conclusion

Tissue plasminogen activator (tPA) as an enzyme, plays a specific role in the fibrinolytic system and it is now established that assaying for tp-A will help in assessing health and prognosis of pregnancy. This study provides valuable insights into the dynamics of fibrinolytic activity during pregnancy in primigravidae and multigravidae. Future research with larger sample sizes and diverse populations could further elucidate the observed differences. Additionally, exploring the potential clinical implications of these findings on pregnancy outcomes and complications would be of significant value.

Acknowledgement

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Conflict Of Interest

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this paper.

Data Availability Statement

The datasets generated and analyzed during this study are not publicly available but are available from the corresponding author upon request.

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