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PAI-1 and tPA as markers of severity among pre-eclamptics in a tertiary institution in north central Nigeria

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Abstract

Objectives: Endothelial dysfunction contributes to the pathogenesis of pre-eclampsia as well as increased production of some factors such as tissue plasminogen activator (tPA) and plasminogen activator inhibitor type-1 (PAI-1). These factors are said to be biomarkers of pre-eclampsia but their role in assessing the severity of pre-eclampsia is under-reported.

Methodology: A cross-sectional study involving 85 subjects with pre-eclampsia. They were classified clinically as having mild or severe pre-eclampsia using ACOG classification. Blood and urine samples were collected for determining tPA, PAI-1, D-dimer and proteinuria in the two groups. Clinical and laboratory values were compared using the IBM®SPSS 20.0 (2011) soft ware packages.

Results: The mean age of the respondents was 29.9±5.2 years. Forty-five (52.9%) of the subjects had severe pre-eclampsia while 40(47.1%) had mild pre-eclampsia. The median values of tPA and PAI-1 of subjects with severe pre-eclampsia were significantly higher than the corresponding values in subjects with mild pre-eclampsia (each p=0.001). There was a positive correlation between each of the tPA and PAI-1 levels with the degree of severity of pre-eclampsia (p=0.001 each).

Conclusion: Fibrinolytic proteins like tPA and PAI-1 are useful in assessing the severity of pre-eclampsia.

Keywords: *Severe pre-eclampsia; fibrinolytic proteins, D-dimer, PAI-1,tPA.*

Résumé

Objectifs: La dysfonction endothéliale contribue à la pathogénèse de la pré-éclampsie, ainsi que la production accrue de certains facteurs, tels que l'activateur tissulaire du plasminogène (tPA) et le type 1 d'inhibiteur de l'activateur du plasminogène (PAI-1). Ces facteurs sont censés être des biomarqueurs de la pré-éclampsie, mais leur rôle dans l'évaluation de la gravité de la pré-éclampsie est sous-déclaré.

Méthodologie: Une étude transversale comprenant 85 sujets avec pré-éclampsie. Elles ont été classées cliniquement comme ayant pré-éclampsie légers ou graves en utilisant la classification ACOG. Des échantillons de sang et d'urine ont été prélevés pour la détermination du tPA, PAI-1, D-dimère et la protéinurie dans les deux groupes. Les valeurs clinique et laboratoire ont été comparés en utilisant l'emballage logistique IBM®SPSS 20,0 (2011).

Résultats: L'âge moyen des répondantes était de 29,9 ± 5,2 ans. Quarante-cinq (52,9%) des sujets avaient pré-éclampsie sévère, tandis que 40 (47,1%) avaient une pré-éclampsie légère.

Les valeurs médianes de tPA et de PAI-1 des sujets avec pré-éclampsie sévère étaient significativement plus élevés que les valeurs correspondantes chez les sujets présentant avec une pré-éclampsie légère (chaque p = 0,001). Il y avait une corrélation positive entre chacun des tPA et PAI-1 avec le degré de gravité de la pré-éclampsie (p = 0,001 chacun).

Conclusion: Les protéinesfibrinolytiques comme le tPA et le PAI-1 sont utiles pour évaluer la gravité de la pré-éclampsie.

Mots-clés: *Pré-éclampsie sévère; protéines fibrinolytiques, D-dimère, PAI-1, tPA.*

Introduction

Pre-eclampsia toxaemia (PET) is a disease of pregnancy occurring after twenty weeks gestation, and characterized by blood pressure of 140/90mmHg or above on two occasions about 4-6hours apart with a preceding normal blood pressure, significant proteinuria (300mg in 24hours) which all resolve completely by 6 weeks postpartum [1]. Pre-eclampsia is a common medical disorder of pregnancy and it involves several organs [2,3]. In the United States, it has an incidence of 2-6% in

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healthy, nulliparous women, while in developing nations the incidence is reported to be between 4-18%. A report from Unilorin Teaching Hospital puts the incidence at about 5-8% [4].

It can be mild or severe. American College of Obstetricians and Gynaecologists (ACOG) Practice bulletin defines severe or mild PET depending on the presence or absence of headache, epigastric tenderness, visual disturbances, seizures and diastolic pressure > 110mmHg, proteinuria, excretion of 5g or more of protein in a 24hr urine specimens or 3+ or greater on two random samples collected four hours apart, and or thrombocytopaenia [5].

The haemostatic changes as well as the overall clinical syndromes of pre-eclampsia occur because of activation of or damage to endothelial cells with resultant fibrin deposition in microvasculature [6,7]. Other components of the syndrome like glomeruloendotheliosis with proteinuria, DIC with elevated D-dimer [8] and hepatic necrosis with increased liver enzymes are due to the activation of endothelial cells [9-11].

Also the biochemical evidence of this endothelial cell damage or alteration is provided by elevated plasma level of endothelial derived factors like, fibronectin, tissue plasminogen activator (tPA), Plasminogen activator inhibitor type-1 (PAI-1), and von-Willebrand factor (vWF) [12,13].

Thus, some of these factors are said to be biomarkers of pre-eclampsia but their role in assessing the severity of pre-eclampsia has not been sufficiently substantiated.

In this study, we aimed to compare these parameters in mild and severe pre-eclamptics and determine whether elevated level of these parameters can predict a severe disease.

Methodology

It was a cross-sectional non-intervention study that included 85 subjects aged 18-42 years. Confirmed pre-eclamptic patients who were yet to be delivered of their pregnancies and had given their consent were serially recruited.

Subject selection and method

Pre-eclampsia was diagnosed according to the criteria of the National Blood Pressure Education Program Working Group as blood pressure of 140/90mmHg and above and confirmed proteinuria (0.3g/L/24h) with or without previous evidence of an underlying hypertensive disorder [5]. However, pregnant women with arterial or venous thrombosis, post-operative and trauma cases, significant medical disorders such as, diabetes mellitus, chronic renal failure, those on any form of anticoagulant therapy and those who declined consent were excluded from the study.

Ethical clearance was obtained from the Ethics and Research Committee of the University of Ilorin Teaching Hospital. A written informed consent was obtained from the patients after explaining the aim of the study and the procedure involved. Symptoms of severe pre-eclampsia like headache, visual disturbances, upper abdominal pain and abnormal bleeding were asked and recorded in the proforma. Clinical signs such as jaundice, paedal oedema, epigastric tenderness and blood pressure were also recorded.

Each subject was grouped as mild or severe pre-eclampsia using the criteria in the American College of Obstetricians and Gynaecologists (ACOG) Practice bulletin [5]. Blood samples were obtained after diagnosis and before patients were delivered of their pregnancies. Eight and half millilitres (ml) of venous blood was taken by standard sterile procedure. Four and half ml out of the whole blood was put into plastic tubes containing half ml sodium citrate(3.8%), and four ml was dispensed into a bottle containing ethylene diamine tetra acetic acid(EDTA). Both bottles were gently mixed to allow proper mixture of the blood sample with the anticoagulant.

The sample in the citrate bottle was centrifuged for 20mins at 3500g to obtain a platelet-poor supernatant, and the supernatant plasma stored at -80^{0}C until assayed. The blood in the EDTA bottle was used for the estimation of packed cell volume (PCV), platelet count within two hours of collection. Prior to obtaining the blood sample, a urine sample from each patient was obtained in a universal plain bottle and analysed immediately.

Laboratory procedures

The following tests were carried out on the blood samples: PCV, Platelet count, Prothrombin time, Activated partial thromboplastin time (aPTT), D-dimer assay, Tissue plasminogen activator, Plasminogen activator inhibitor-type 1. Urinalysis to quantify proteinuria via the dipstick method was carried out on the urine samples. The platelet count and PCV were determined using the *sysmex KX 21*^{x} *(Sysmex Corporation, Kobe, Japan)* automated cell counter. Prothrombin time was determined using commercially prepared reagents based on the one stage test of Owren. Activated partial thromboplastin time was determined using commercially prepared

reagents based on the method of Proctor and Rapaport.

Tissue plasminogen activator was assayed via the AssayMax Human tPA ELISA kit *(Product of Saint Charles, Missouri, USA)*. Plasminogen activator inhibitor type-1 (PAI-1) was assayed via the AssayMax Human PAI-1 ELISA kit *(Product of Saint Charles, Missouri, USA)*. D-DIMER ASSAY was assayed with the TECHNOZYM D-dimer ELISA kit *(Product of Technozyme, Austria)*.

Data analysis

Data was analyzed using the IBM® SPSS version 20.0 (*IBM corporation, Virgina, USA*) 2011 for windows software package. After the generation of frequency tables and simple proportions, Student's *t*-tests and Mann-Whitney *U* tests were used to identify significant differences for normally distributed continuous variable and the skewed data respectively. However, the skewed data were not transformed before or after analysis.

In determining the correlation between severity of pre-eclampsia and plasma level of the proteins measured in the laboratory, Spearman's rank correlation was used. While the correlation between various laboratory parameters were determined using the Pearson's correlation test. Binary logistic regression was used to determine the predictor of severity among the major laboratory parameters measured. Also, a receiver's operating characteristic curve was used to determine the sensitivity of these laboratory tests in determining the severity of pre-eclampsia.

Result

Eighty five pregnant women with pre-eclampsia between the ages of 18-42 years were recruited into this study, the mean age was 29.9±5.2. Majority of them (97.6%) were married. Also, 20% of the subjects were in their second trimester while 80% were in their third trimester. 42.4% of the subjects were primigravida, 32.9% were multigravida and only 24.7% were granmultip as shown in table 1. However, forty-five (52.9%) of the subjects had severe pre-eclampsia while 40(47.1%) had mild pre-eclampsia.

There were significant differences in values of PAI-1 and tPA among mild and severe pre-eclamptics p=0.001 and <0.001 respectively. Also, there was a significant positive correlation between the severity of pre-eclampsia and each of the plasma levels of PAI-1 and tPA p=0.001 and <0.001 respectively.

Table1: Reproductive characteristics of respondents

Variable	Subjects n(%)
Age group (Years)	
≤18	2(2.4)
19-35	72(89.7)
>35	11(12.9)
Range	18-42
Mean±SD	29.9±5.2
Gestational age (Weeks)	
20-26	17(20.0)
27-39	68(80.0)
Parity	
Primigravida	36(42.4)
Multigravida	28(32.9)
Granmultip	21(24.7)

Furthermore, the risk of developing severe pre-eclampsia was two folds higher in subjects with high PAI-1 level (1.23-4.28ng/ml at 95% CI) and *p=0.009*.

In this study, the following parameters showed positive correlation, D-dimer (ng/ml) and tPA (ng/ml) r=0-605, p=0.001. D-dimer (ng/ml) and proteinuria r=0.247, p=0.030. tPA (ng/ml) and PAI-1 (ng/ml) r=0.300, p=0.008. tPA (ng/ml) and Proteinuria r=0.248, p=0.030, PAI-1 (ng/ml) and diastolic pressure r=0.330, p=0.003. However,

Table 2: Comparison of Fibrinolytic Proteins and Platelet count in mild and severe pre-eclamptics

Variable	Mild eclampsia n=40	pre-Severe eclampsia n=45	pre-Mann Whitney U	p-value
D-dimer (ng/ml)				
Median	1020.00	1220.000	687.000	0.602
tPA (ng/ml)				
Median	0.075	0.200	350.000	<0.001
PAI-1 (ng/ml)				
Median	3.250	4.050	419.000	0.001
Platelet count (x 10^9/L)				
Median	183.000	150.000	621.000	0.232

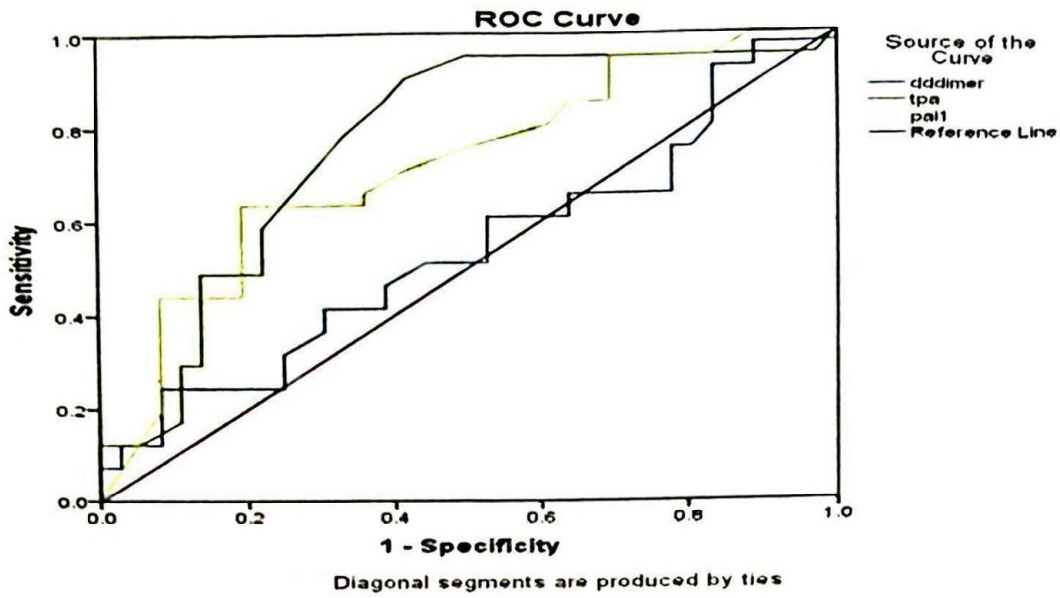

Fig.1: Receiver' Operation Characteristic curve for determining the Sensitivity of tPA, PAI-1 and D-dimer as markers of severity of Pre-eclampsia in the laboratory

negative correlation was found between platelet count and proteinuria r=-0.229, p=0.046. Also platelet count and diastolic pressure had a negative correlation r=-0.250, p=0.024.

Receiver's Operation Characteristic curve for determining the Sensitivity of tPA, PAI-1 and D-dimer as markers of severity of Pre-eclampsia in the laboratory showed that the area under the curve for tPA, PAI-1 and D-dimer were 0.763, 0.716 and 0.535 respectively (95%CI: 0.65-0.88 (ng/ml) for tPA, 0.6-0.8 (ng/ml) for PAI-1 and 0.4-0.63 for D-dimer respectively). However, p=0.004 and 0.003 respectively for tPA and PAI-1 only.

Discussion

It is known that the pathogenesis of pre-eclampsia may be associated with endothelial cell dysfunction that tends to increase the expression of endothelial-derived tPA, its inhibitor(PAI-1) as well as fibrinolytic products(D-dimer) in pre-eclamptic subjects. This theory is supported by the finding in the present study in which plasma levels of endothelially derived tPA and PAI-1 were significantly higher in severe pre-eclamptics than mild pre-eclamptics. Similar findings were reported by some researchers [14-16] but in contrast, Declerck *et al* reported that PAI-1 level of several pre-eclamptic patients were not different from those obtained in normal pregnancy [17]. The disparity of Declerck study may be attributed to the subjects used(normal pregnancy and pre-eclamptics) as well as the time of specimen collection in view of the diurnal variation of PAI-1 level with an early morning peak [8].

There was a significant positive correlation between severity of pre-eclampsia and values of tPA and PAI-1, a finding which is similar to a report by Yuditiya [15]. This shows that the more the endothelial cell dysfunction, the more the factors produced, the more the severity of the disease and presence of some clinical symptoms.

Equally noteworthy in the current study is the insignificant difference between D-dimer values of mild and severe pre-eclamptics which is in contrast to reports of some studies [18,19]. The insignificant difference in the index study may suggest that fibrinolysis only increases up to a point in pre-eclampsia when fibrinolytic failure now occurs. The above finding is further buttressed by the insignificant correlation between D-dimer and severity of pre-eclampsia. This finding is in contrast to the study of Javadi *et al* [20], where D-dimer correlated with the degree of severity.

There are a number of experimental observations supporting the hypothesis that PAI-1 is the principal physiologic inhibitor of tPA and a primary regulator of vascular fibrinolysis [17,21,22]. The finding in the present study has also supported this fact as both tPA and PAI-1 increased and showed a significant positive correlation.

The tPA protein acts on plasminogen to form plasmin which acts on fibrin to form D-dimer which shows that as intravascular coagulation is occurring, the fibrinolytic system is being activated. Thus,

supporting the significant positive correlation between tPA and D-dimer found in the present study. This finding is also similar to the finding in the study by Luis *et al* [23].

Furthermore, the increased endothelial disturbance and resultant glomeruloendotheliosis increases both the tPA and proteinuria levels respectively and explains the correlation of tPA with proteinuria, a known marker of severe pre-eclampsia. The significant correlation between proteinuria and D-dimer shows that the more the intravascular coagulation, the more the fibrinolysis and the more the proteinuria levels. PAI-1 also correlated positively with diastolic pressure in the index study showing that increased PAI-1 level is associated with increased diastolic pressure.

The insensitivity of D-dimer in determining the severity of pre-eclampsia, could be due to the fact that increased level of D-dimer can be as a result of other factors like inflammation [8]. However, the sensitivity of tPA and PAI-1 in determining the severity of pre-eclampsia further buttresses the link between endothelially derived factors and pre-eclampsia.

In this study, the best laboratory predictor of severe pre-eclampsia was PAI-1, a finding which is similar to the earlier reported finding by Astedt [24]. In fact, the odds of having severe PET is about 2.3 folds higher in patients with high PAI-1. The ability of PAI-1 level to predict severe pre-eclampsia suggest that severe PET is a state of fibrinolytic failure as found in a previous study [25]. It also shows that it predominates in severe PET and therefore fosters the deposition of fibrin, thus, explains the complication associated with the disease. The predominance of PAI-1 could be as a result of the increased stimulatory effect of angiotensin II on PAI-1 in addition to the endothelial dysfunction. The rise in angiotensin II, which is even more at the placenta bed of a pre-eclamptic woman [26], is as a result of activation of renin angiotensin system due to increased estrogen level in pregnancy. However, D-dimer was not useful in predicting the severity of pre-eclampsia and from this study, the predictive value of tPA could only be substantiated using a larger population.

In conclusion, PAI-1 and tPA are useful in assessing severity of pre-eclampsia in the laboratory, and, severe pre-eclampsia may be a state of fibrinolytic failure.

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Contribution

All authors contributed to the study protocol, data collection and the correspondence author did the final draft of the paper.

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