

Neutrophil gelatinase-associated lipocalin and Endothelin-1 as markers for the onset and severity of preeclampsia

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Background: The onset of preeclampsia (PE) may be early (EOPE), or late (LOPE). Preeclampsia may also be characterized by severe features (PS) or without severe features (PNS). This study aimed to predict the onset and severity of PE using biomarkers including neutrophil gelatinase-associated lipocalin (sNGAL) and endothelin-1 (ET-1).

Method: This was a case-control study from January to June 2020. The study involved 270 pregnant women (control=135, PE=135), aged between 18-37 years. The cases and controls were matched by maternal and gestational age at sampling. Serum samples were assayed for sNGAL and ET-1 using the ELISA technique.

Results: Serum NGAL and ET-1 were significantly increased in PE and also increased in PE characterized by severe features ($P<0.010$). Both sNGAL and ET-1 positively correlated with blood pressure in PE ($P<0.050$). In predicting PE, the area under the curve, sensitivity and specificity of sNGAL were 0.76, 46.2% and 97.8% while that of ET-1 were 0.82, 66.8%, and 92.5% respectively. Also, ET-1 could differentiate PS from PNS with a sensitivity of 85.7% and specificity of 82.1%.

Conclusion: Serum ET-1 is better than sNGAL in the prediction of PE. We recommend screening pregnant women using ET-1 for the early detection and management of PE.

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INTRODUCTION

Preeclampsia is the new-onset of hypertension in pregnancy after 20 weeks of gestation. Globally, PE affects between 3-8% of all pregnancies and it is among the leading cause of maternal and infant morbidity and mortality in Sub-Saharan Africa (Lugobe *et al.*, 2020). Clinically, there are two types of PE; early-onset PE (EOPE), occurring before 34 weeks and late-onset PE (LOPE), occurring at or after 34 weeks of gestation. Preeclampsia may also be characterized by severe features (PS) or without severe features (PNS). Although the aetiopathology

of PE is unknown, it has however been associated with aberrant trophoblastic invasion, placental hypoxia, oxidative stress and endothelial injury leading to renal, hepatic and cardiovascular systems dysfunction (Alese *et al.*, 2021; Atiba *et al.*, 2016; Ives *et al.*, 2020).

In response to the endothelial cell damage, it has been suggested that there is a direct secretion of sNGAL into damaged renal tubular cells with the intent of repair since sNGAL has been shown to induce re-epithelialization (Simonazzi *et al.*, 2015).

Serum NGAL has been suggested as a better predictor of renal damage than creatinine (CRT). Various studies have found elevated levels of sNGAL in PE with some reporting a significant correlation between sNGAL and the severity of the disease's symptoms (Artunc-Ulkumen *et al.*, 2015; Kim *et al.*, 2013; Sachan *et al.*, 2014; Scazzochio *et al.*, 2014).

The generalized inflammation, angiogenic imbalances (Nabweyambo *et al.*, 2021) and increased blood pressure in PE also affect serum levels of ET-1, a natural vasoconstrictor, synthesized, stored and secreted by a range of cells, including Weibel-Palade bodies of endothelial cells and the syncytiotrophoblasts of the placenta (Langeza Saleh *et al.*, 2016). From previous studies, serum levels of ET-1 were elevated in PE as compared to the controls and the levels increased with the severity of the symptoms of the disease (Aggarwal *et al.*, 2012; Gunilla Ajne *et al.*, 2003; Baksu *et al.*, 2005; George and Granger, 2011; Nishikawa *et al.*, 2000; Taylor *et al.*, 1990; Wantania *et al.*, 2021). However, concerns regarding the interpretation of serum ET-1 results have been raised: firstly, ET-1 is an auto-crine/paracrine factor whose serum concentration does not reflect local tissue production (George, Palei and Granger, 2012); secondly, ET-1 blood levels may suffer from haemodilution (Coffey, 2019).

Although the aetiopathology of PE is unknown, there are population and sub-population variabilities in the prevalence, onset and severity largely due to genetic and environmental factors (Y. Cui *et al.*, 2012; Rana *et al.*, 2019). Similarly, changes in serum biochemical markers of PE may also vary due to influences from similar factors. There is therefore the need for population-specific studies to establish local reference data for early PE detection. The study, therefore, aims to predict the onset and severity of PE using serum levels of NGAL and ET-1 in the Ghanaian population.

MATERIALS AND METHODS

Study design and setting

This was a case-control study from January to June

2020. The study was conducted at the Richard Novarti Catholic Hospital, located at Sogakope in the South Tongu District of the Volta Region of Ghana.

Participants

The study involved 270 pregnant women (controls=135, and PE=135). The cases were further stratified based on the onset of PE as either early-onset PE [EOPE (n=71)] or late-onset PE [LOPE (n=64)] and also based on the presence of severe features [PS (n=48)] or the absence of severe features [PNS (n=87)]. The definition of PE, its onset and its severity was based on the guidelines of the American College of Obstetricians and Gynecologists (ACOG, 2019). Preeclampsia was defined as the new-onset of hypertension (systolic ≥ 140 mmHg, diastolic ≥ 90 mmHg, taken 4 hours apart) after 20 weeks of gestation with proteinuria (≥ 30 mg/dL protein per 24-hr urine collection or ≥ 0.30 protein/CRT ratio or urine protein (2+) on the dipstick reading at presentation). In the absence of proteinuria, the following criteria was used: thrombocytopenia (PLT $< 100 \times 10^9$ /L); renal insufficiency (serum CRT > 1.1 mg/mL); impaired liver function (≥ 2 x level of transaminases).

Early-onset PE was defined as PE occurring before 34 weeks of gestation and LOPE was defined as PE occurring at/after 34 weeks of gestation. Preeclampsia with severe features was defined as PE characterized by systolic ≥ 160 mmHg or diastolic ≥ 110 mmHg blood pressure plus all other manifestations of PE. PNS was defined as PE without severe features. The inclusion criterion was a diagnosis of PE, whether early or late-onset, with or without severe features. Exclusion criteria included all women with twin gestations, those previously diagnosed with chronic hypertension, sickle cell anaemia, diabetes mellitus, gestational diabetes, cardiovascular disorder, renal disease and those who were on antihypertensive or magnesium medication before the recruitment. Sociodemographic and clinical information was collected using a structured questionnaire and also from their medical records.

Variables

The main predictor variables included serum NGAL and ET-1. The covariables included sociodemographic and clinical history (age, BMI, proteinuria, parity, gravidity, obstetric history etc.), fasting lipids [total cholesterol (TCHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TRIG)], liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transaminase (GGT)] and creatinine (CRT). The controls and cases differed in the BMI which may be a confounding factor and was therefore controlled as appropriate.

Measurements

Blood pressure was measured with the aid of a cuff sphygmomanometer according to the fifth Korotkoff sound. Urine screening for proteinuria was performed using a urine dipstick. A single peripheral venous blood sample was collected from each subject's antecubital vein after an overnight fast (12 h) into an EDTA and a gel separator tube. The serum samples were allowed to clot for 30 min at 4°C and both tubes were then centrifuged at 1500 rpm for 10 min to separate the serum/plasma. Serum and plasma samples were aliquoted into plastic cryotubes and then frozen at -25°C until analysis.

Serum NGAL was analysed in duplicates using the ELISA kit from ABCAM (152 Grove Street Waltham, MA 02453, USA). The kit had a sensitivity of 14.8 pg/mL, within a measurable range of 46.9 pg/ml - 3000 pg/ml. The intra-assay and inter-assay coefficients of variation were $\leq 3.8\%$ and $\leq 2.7\%$ respectively. Serum ET-1 were analyzed in duplicates using Human ELISA kit (Biomedica Medizinprodukte GmbH, Divischgasse 4, 1210 Vienna, Austria). The sensitivity of the ET-1 ELISA kit was 0.086 pg/mL within a detectable range of 0.0 - 12.85 pg/mL. The intra-assay and the inter-assay coefficient of variations were $\leq 4\%$ and $\leq 5\%$ respectively. The total cholesterol (TCHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TRIG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and CRT

were assayed using the plasma/serum samples (as appropriate) through routine biochemistry analysis on the BT 1500 automated biochemistry analyzer (Biotechnica Instruments, SPA, Italy) following the manufacturer's instructions and using the recommended reagents. The samples in this study were not previously thawed and refrozen.

Bias mitigation

To reduce bias and confounders, the cases and controls were matched by maternal and gestational age at the time of sampling. Where there were differences in BMI between the cases and controls, the independent variables were adjusted or controlled for BMI using logistic regression analysis before the results were reported including the p-values.

Statistical analysis

Data were entered into a Microsoft Excel spreadsheet and statistical analysis was performed in SPSS (v23), MedCalc and GraphPad Prism (v8). The normality of the data was checked using the Kolmogorov-Smirnov test. Continuous data were reported as either mean $\pm$ SD or median (IQR) and frequency (%) for categorical variables. Comparison of the means and median values were performed using the student t-test and the Mann Whitney U test respectively (2-tailed). Spearman rank correlation was used to determine the relationship between covariates. Logistic regression analysis was used for odds ratio determinations. Receiver operator characteristics graphs were constructed by plotting the sensitivity against 100-specificity using the Hanley & McNeil method in MedCalc. A probability level of $P < 0.050$ was considered statistically significant.

Ethics declaration

All of the procedures were performed per the approved guidelines laid down in the 1964 Declaration of Helsinki and its later amendments. The study was also approved by the Institutional Review Board (IRB) of the University for Development Studies (UDS), Tamale. All participants gave their written informed consent before the study.

RESULTS

Participants

The study initially enrolled 183 pregnant women with PE seen in the hospital. Of the 183, 19 (10.4%) did not meet the inclusion criteria and were therefore excluded, 10 (5.5%) were lost to follow-up while 19 (10.3%) later declined their participation in the study. In the end, 135 cases were retained in the analysis. In addition, 135 eligible controls from the same hospital, matched for maternal and gestational age were enrolled.

General characteristics of the study population

The BMI, at the time of sampling, was higher in PE ($P<0.001$). A pregnant woman with a history of multigravida [OR: 1.771(1.077-2.915)], and obstetric history including abortion or caesarean section [OR: 4.857(2.562-9.208)] had greater odds of developing PE (Table 1).

Serum NGAL and ET-1 levels in PE

From Table 2, the serum levels of sNGAL and ET-1 and the other biomarkers except GGT were significantly elevated in PE ($P<0.050$). Also, preeclampsia with late-onset was characterized by elevated levels of GGT and CRT ($P<0.050$). From Table 3, PE with severe features had significantly high levels of sNGAL, ET-1 and other biomarkers including TRIG, ALT, AST and GGT ($P<0.010$).

Correlation between sNGAL, ET-1 and covariates

The correlation between sNGAL, ET-1 and other variables is shown in Table 4. There were significant correlations between sNGAL and ET-1 in PE ($r=0.388$, $P<0.010$) and also in LOPE ($r=0.571$, $P<0.001$). Both sNGAL and ET-1 were significantly correlated with diastolic blood pressure in PE ($P<0.001$).

Multivariate logistic regression

Univariate logistic regression analyses were performed for all predictor variables and only significant variables were entered in a multivariate logistic regression analysis (Tables 5). The odds of PE increased significantly per every unit increase in sNGAL, ET-1, AST and CRT ($P<0.050$). Also, the odds of LOPE relative to EOPE increased sig-

nificantly with a unit increase in the levels of GGT and CRT while the odds of PS relative to PNS also increased significantly with every unit increase in the levels of ET-1 and AST ($P<0.050$).

Receiver operator characteristics (ROC)

The significant variables from the multivariate logistic regression analysis were used for ROC analysis (Fig. 1). Using the control as the reference, the AUC of sNGAL and ET-1 were not significantly different (0.76 vs. 0.82, $P=0.136$). However, the AUC of ET-1 was significantly larger than AST (0.82 vs. 0.70, $P=0.003$) and CRT (0.82 vs. 0.67, $P=0.001$). In predicting PE, sNGAL had a sensitivity of 46.2% and a specificity of 97.8% at the cut-off value of 98.0 ng/mL while the sensitivity of ET-1 was 66.8%, and the specificity was 92.5% at a cut-off value of 37.0 pg/mL. There were no significant differences in AUC between GGT and CRT in the prediction of LOPE (0.66 vs. 0.64, $P=0.792$). Also, in the prediction of PS, relative to PNS, the AUC did not differ significantly between ET-1 and AST (0.81 vs. 0.90, $P=0.066$), however, the sensitivity of ET-1 was 85.7%, and a specificity of 82.1% at a cut-off value of 84.0 pg/mL.

DISCUSSION

The study aimed to predict the onset and severity of PE using sNGAL, ET-1 and other biomarkers. Serum levels sNGAL and ET-1 were significantly increased in PE and also in PS. Both sNGAL and ET-1 were independent determinants of PE but only ET-1 could independently predict PS relative to PNS. Serum NGAL was significantly correlated with ET-1 in PE and with CRT in EOPE, all with a medium effect size. Both sNGAL and ET-1 levels correlated positively with diastolic blood pressure in PE. Serum ET-1 was better than sNGAL in predicting PE and its severity.

The levels of sNGAL were significantly elevated in PE relative to the controls. This was consistent with previous studies (Akbas and Koyuncu, 2020; Artunc-Ulkumen *et al.*, 2015; L. Cui *et al.*, 2018; Rosario D'Anna *et al.*, 2010; Jiang *et al.*, 2021; Karampas *et al.*, 2014; Kim *et al.*, 2013; Scazzocho *et al.*, 2014; Sorohan *et al.*, 2020). Also, PE with se-

Table 1. The general characteristics of the study population at sampling

Variable	Control, n (135)	PE, n (135)	cOR(95%CI)/P-value
Age (yrs)	27.8±4.63	27.9±4.40	0.893
Gestational age (weeks)	29.7±3.02	29.9±3.00	0.614
Weight	67.8±15.26	76.9±15.84	<0.001
Height	156.5±7.04	159.78.59	0.001
BMI	27.4±5.83	30.2±6.14	<0.001
Age (yrs)			
18-25	50(52.1)	46(47.9)	1
26-30	47(47.5)	52(52.5)	1.203(0.683-2.110)
>30	38(50.7)	37(49.3)	1.058(0.578-1.937)
Gestational age (weeks)			
20-28	62(54.4)	52(45.6)	1
29-31	26(41.3)	37(58.7)	1.697(0.911-3.162)
>30	47(50.5)	46(49.5)	1.167(0.674-2.020)
BMI (kg m⁻²)			
<25	54(69.2)	24(30.8)	1
25-30	45(46.9)	51(53.1)	1.067(0.570-1.997)
>30	36(37.5)	60(62.5)	2.051(1.080-3.896) *
Ethnicity			
Ewe	102(47.9)	111(52.1)	1
Non-Ewe	33(57.9)	24(42.1)	0.668(0.370-1.206)
Marital status			
Married	75(41.0)	108(59.0)	1
Not married	60(71.4)	24(28.6)	0.278(0.159-0.485) ***
Education			
None/primary	48(50.0)	48(50.0)	1
Secondary	72(51.1)	69(48.9)	0.958(0.0570-1.610)
Tertiary	15(45.5)	18(54.5)	1.200(0.543-2.653)
Employment status			
Unemployed	48(72.7)	18(27.3)	1
Self-employed	72(43.6)	93(56.4)	3.444(1.847-6.422) ***
Salary worker	15(38.5)	24(61.5)	4.267(1.838-9.907) **
Parity			
Nulliparous	60(55.6)	48(44.4)	1
Primiparous	27(42.9)	36(57.1)	1.667(0.890-3.120)
Multiparous	48(48.5)	51(51.5)	1.328(0.768-2.295)
Gravidity			
Primigravida	60(58.8)	42(41.2)	1
Multigravida	75(44.6)	93(55.4)	1.771(1.077-2.915) *
Obstetric history			
None	120(58.8)	84(41.2)	1
Abortion/cs/others	15(22.7)	51(77.3)	4.857(2.562-9.208) ***
Family history			
None	93(46.3)	108(53.7)	1
HPT/DM	42(60.9)	27(39.1)	0.554(0.317-0.967) *

*Results were presented as mean ± SD for parametric and n (%) for categorical variables. *Significant at P<0.050 level
 significant at P< 0.010 level, *significant at P< 0.001 level (unpaired, 2-tailed)*

vere features were characterized by elevated levels of sNGAL as has been demonstrated in previous studies (Artunc-Ulkumen *et al.*, 2015; Kim *et al.*, 2013; Sachan *et al.*, 2014; Scazzochio *et al.*, 2014) but contrary to studies by Simonazzi *et al.* (2015) and L. Cui *et al.* (2018) who did not find significant differences in sNGAL levels between PNS and PS. Also consistent with this study, Rosario D'Anna *et al.* (2008) did not find significant differences in levels of sNGAL between EOPE and LOPE.

The activation of the endothelium in PE leads to leukocyte stimulation including monocytes and gran-

ulocytes. Serum NGAL has an immune-modulatory property including participation in inflammatory processes in PE (Wiles *et al.*, 2020). Karampas *et al.* (2014) and Rosario D'Anna *et al.* (2010) have shown that sNGAL levels continue to increase across trimesters in pregnancies characterized by PE. Serum NGAL is highly accumulative in blood especially after an ischemic or nephrotoxic assault on the kidney unlike CRT and other biomarkers (Patel *et al.*, 2013). This study found a significant correlation between sNGAL and CRT in EOPE. According to Artunc-Ulkumen *et al.* (2015), and Scazzochio *et al.* (2014), serum NGAL indicates renal injury early

Table 2. Comparison of predictor variables stratified by the onset of preeclampsia

Variable	Control, n (135)	PE, n (135)	EOPE, n (71)	LOPE, n (64)
sNGAL (ng/mL)	79.1(72.6-85.4)	95.2(78.6-116.9) **	84.9(78.6-101.7)	108.8(82.5-119.1)
ET-1 (pg/mL)	17.9(10.0-27.8)	60.0(30.0-146.0) **	59.0(30.0-134.0)	82.0(46.0-148.0)
TCHOL (mmol/L)	3.5(2.8-4.7)	4.6(3.5-5.1) **	4.2(3.6-5.1)	4.7(3.4-5.6)
HDL (mmol/L)	1.3(1.0-1.5)	1.0(0.5-1.5) *	1.1(0.6-1.6)	1.2(0.5-1.4)
LDL (mmol/L)	2.1(1.5-2.7)	2.8(1.7-3.9) **	2.8(2.1-3.9)	3.4(1.7-4.3)
TRIG (mmol/L)	0.8(0.5-1.1)	0.9(0.7-1.3) **	1.0(0.7-1.2)	0.9(0.7-1.4)
ALT (IU/L)	11.1(6.2-22.5)	23.7(16.3-56.1) **	25.7(14.2-53.8)	21.8(12.2-56.1)
AST (IU/L)	11.2(6.1-23.0)	22.1(10.6-45.0) **	18.1(9.2-45.1)	25.2(12.5-39.5)
GGT (IU/L)	21.4(11.3-44.0)	16.7(10.6-43.7)	19.5(10.4-71.9)	15.8(7.0-39.2) †
CRT (µmmol/L)	48.1(36.6-56.0)	61.3(44.9-91.3) **	65.0(46.3-92.7)	56.3(44.9-69.5) †

Results were presented as median (IQR). Pregnant women in the control group were compared with preeclamptic women (PE) while early-onset preeclamptic women (EOPE) were compared to late-onset preeclamptic women (LOPE) using the Mann-Whitney U test (2-tailed)

*Significant at the $P<0.010$ level, **significant $P<0.001$ level compared to control

†Significant at the $P<0.050$ level compared to EOPE

Table 3. Comparison of predictor variables stratified by the severity of preeclampsia

Variable	Control, n (135)	PE, n (135)	PNS, n (87)	PS, n (48)
sNGAL (ng/mL)	79.1(72.6-85.4)	95.2(78.6-116.9) **	84.3(77.3-113.0)	132.5(100.2-139.1) ‡
ET-1 (pg/mL)	17.9(10.0-27.8)	60.0(30.0-146.0) **	44.0(24.5-72.0)	136.0(106.0-170.0) ‡
TCHOL (mmol/L)	3.5(2.8-4.7)	4.6(3.5±5.1) **	4.6(3.3-5.1)	4.7(3.7-5.3)
HDL (mmol/L)	1.3(1.0-1.5)	1.0(0.5-1.5) *	1.1(0.7-1.5)	1.0(0.3-1.4)
LDL (mmol/L)	2.1(1.5-2.7)	2.8(1.7-3.9) **	2.8(1.7-3.9)	2.7(1.7-4.7)
TRIG (mmol/L)	0.8(0.5-1.1)	0.9(0.7-1.3) **	0.9(0.7-1.2)	1.1(0.7-1.6) †
ALT (IU/L)	11.1(6.2-22.5)	23.7(16.3-56.1) **	19.3(10.4-25.1)	67.9(35.4-78.5) ‡
AST (IU/L)	11.2(6.2-23.0)	22.1(10.6-45.0) **	12.7(9.2-35.2)	35.6(23.1-52.3) ‡
GGT (IU/L)	21.4(11.3-44.0)	16.7(10.6-43.7)	14.4(10.9-36.5)	36.2(10.3-56.2) †
CRT (µmmol/L)	48.1(36.6-56.0)	61.3(44.9-91.3) **	60.2(45.1-89.0)	67.4(36.6-102.1)

Results were presented as median (IQR). Pregnant women in the control group were compared with preeclamptic women (PE) while preeclamptic women with severe features (PS) were compared to preeclamptic women without severe features (PNS) using the Mann-Whitney U test (2-tailed)

*Significant at $P<0.010$ level, **significant $P<0.001$ level compared to control

†Significant at $P<0.010$ level, ‡Significant at $P<0.001$ level compared to PNS

Table 4. Spearman rank correlation matrix between NGAL, EDN-1 and covariates

Variable	sNGAL									
	Control	PE	EOPE	LOPE	PNS	PS	Control	PE	EOPE	LOPE
sNGAL (ng/mL)	1	1	1	1	1	1	0.074	0.388**	0.289	0.571**
ET-1 (pg/mL)	0.074	0.388**	0.289	0.571**	0.264*	-0.238	1	1	1	1
TCHOL (mmol/L)	0.043	0.246**	0.448**	0.320*	0.327**	0.250	0.166	0.112	0.382**	-0.134
HDL (mmol/L)	-0.265**	0.113	-0.072	0.429**	0.304**	-0.100	0.212*	0.103	0.034	0.253
LDL (mmol/L)	0.085	0.096	0.365**	0.025	0.041	0.167	-0.165	0.064	0.360**	-0.215
TRIG (mmol/L)	-0.117	0.378**	0.455**	0.481**	0.363**	0.441*	0.062	0.065	0.162	0.047
ALT (IU/L)	-0.095	0.086	0.146	-0.014	-0.116	0.357	0.328**	0.166	0.022	0.086
AST (IU/L)	-0.087	0.247**	0.123	0.221	-0.161	0.533**	0.186	0.378**	0.448**	0.247
GGT (IU/L)	-0.076	0.257**	0.567**	-0.164	0.224*	-0.233	-0.060	0.162	0.199	0.189
CRT (μmol/L)	0.013	-0.019	0.480**	-0.404**	0.121	-0.183	0.074	-0.166	0.173	-0.154
SBP (mmHg)	0.066	0.068	-0.173	0.573**	-0.185	0.075	0.064	0.430**	0.226	0.543**
DBP (mmHg)	-0.138	0.323**	0.239	0.424**	-0.019	0.114	0.012	0.410**	0.463**	0.431**

Results were presented as Spearman Rank correlation coefficient (r). *Correlation is significant at $P < 0.050$ level (2-tailed), **Correlation is significant at $P < 0.010$ level (2-tailed). Boldface r = Spearman rank correlation coefficient with a medium effect size ($0.30 \leq r \leq 0.50$), boldface and underlined r = Spearman rank correlation coefficient with a large effect size ($r > 0.50$).

Table 5. Independent factors associated with the onset and severity of preeclampsia based on multivariate analysis

Variable	Control(ref) vs. PE		EOPE (ref) vs. LOPE		PNS (ref) vs. PS	
	aOR (95%CI)	#	P-value	aOR (95%CI)	P-value	aOR (95%CI)
sNGAL (ng/mL)	1.085(1.040-1.131)		<0.001	-	-	1.050(0.995-1.108)
ET-1 (pg/mL)	1.095(1.056-1.135)		<0.001	-	-	1.024(1.006-1.043)
TCHOL (mmol/L)	0.574(0.195-1.688)		0.313	-	-	-
HDL (mmol/L)	0.249(0.047-1.329)		0.104	-	-	-
LDL (mmol/L)	2.375(0.806-7.002)		0.117	-	-	-
TRIG (mmol/L)	0.660(0.082-5.297)		0.696	-	-	31.636(0.236-4239.124)
ALT (IU/L)	1.022(0.978-1.067)		0.335	-	-	1.136(0.978-1.320)
AST (IU/L)	1.042(1.009-1.076)		0.012	-	-	1.063(1.019-1.108)
GGT (IU/L)	-		-	0.979(0.962-0.996)	0.019	1.058(0.972-1.151)
CRT (μmol/L)	1.085(1.047-1.125)		<0.001	0.984(0.970-0.999)	0.036	-

Results were presented as adjusted odds ratios (aOR, 95%CI). The cells left blank (-) were variables that were not significant in the univariate analysis. In differentiating between PE and controls, EOPE and LOPE, PNS and PS, the control, EOPE and PNS were used as the reference respectively. PE; preeclampsia, EOPE; early-onset preeclampsia, LOPE; late-onset preeclampsia, PNS; preeclampsia with no severe features, PS; preeclampsia with severe features. # Adjusted for BMI

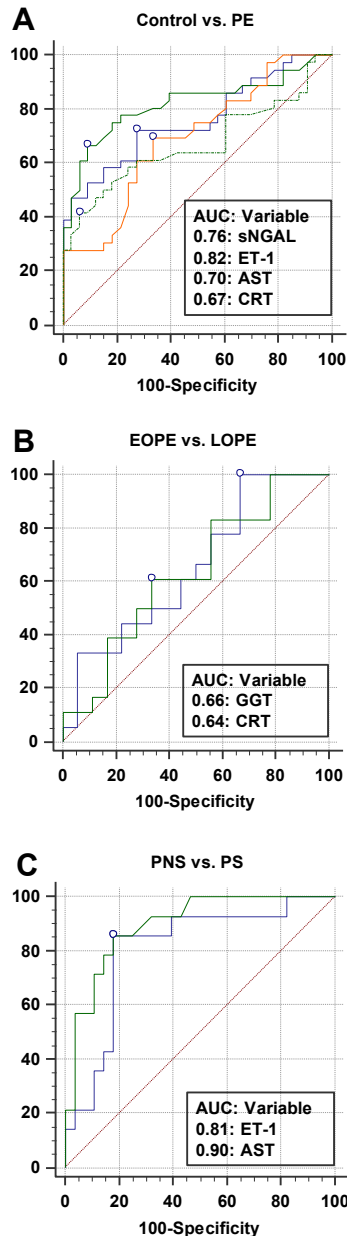


Fig. 1. Receiver operator characteristics plot of sensitivity versus 100-specificity using the Hanly and McNeal technique: plot of controls (ref) vs. preeclampsia (A), the plot of early-onset preeclampsia-EOPE (ref) vs. late-onset preeclampsia-LOPE (B), plot of preeclampsia with no severe features-PNS (ref) vs. preeclampsia with severe features-PS (C).

and more accurately before renal function tests get worsened, and it is, therefore, a suitable candidate for early PE detection. Significant relationships were found between sNGAL and proteinuria (Rosario D'Anna *et al.*, 2010; Kim *et al.*, 2013; Patel *et al.*, 2013; Sachan *et al.*, 2014).

Linear regression analysis by Simonazzi *et al.* (2015), indicated a positive and significant relationship between sNGAL, blood pressure, CRT and proteinuria. Previous studies have shown that renal blood flow and glomerular filtration rates are decreased in PE as shown by the loss of podocytes, endothelial swelling and glomerular endotheliosis in biopsies from PE patients. Damaged endothelial cells in PE may induce clotting, as well as loss of anticoagulant ability as levels of nitric oxide and prostaglandin, plummets leading to kidney thrombotic microangiopathy (Ives *et al.*, 2020; Sani, *et al.*, 2019). The direct relationship between NGAL and proteinuria may stem from the direct excretion of sNGAL into damaged renal tubular cells with the intent of repair since sNGAL can induce re-epithelialization (Simonazzi *et al.*, 2015). Lack of differences in sNGAL between EOPE and LOPE may be due to the early onset of endothelial dysfunction in PE such that sNGAL levels reach their peak by the second half of pregnancy Rosario D'Anna *et al.* (2010).

Serum levels of ET-1 were elevated in PE as compared to the controls and the levels increased with the severity of the symptoms of the disease. This is consistent with previous studies although this is not a universal observation (Aggarwal *et al.*, 2012; Gunilla Ajne *et al.*, 2003; Baksu *et al.*, 2005; George and Granger, 2011; Nishikawa *et al.*, 2000; Taylor *et al.*, 1990; Wantania *et al.*, 2021). Multiple regression analysis showed that ET-1 is an independent determinant of hypertension and proteinuria in PE (Verdonk *et al.*, 2015). Several studies have demonstrated the role of ET-1 in the aetiopathology of PE including the elevated circulating ET-1, increase in ET-1 converting enzyme (ECE) activity, the differential effect of systemic ECE inhibition and increased localized ET-1 production in tissues of maternal origin (Ajne *et al.*, 2005; Gunilla Ajne *et al.*, 2003; Baksu *et al.*,

2005; Faxén *et al.*, 1997; Napolitano *et al.*, 2000; Taylor *et al.*, 1990). Studies have shown increased preproendothelin message levels in tissue beds of PE women suggesting that ET-1 may play an autocrine/paracrine role in PE since local production of ET-1 was significantly increased (Faxén *et al.*, 1997; Napolitano *et al.*, 2000).

In the reduced uterine perfusion pressure (RUPP) model, where blood flow to the uterus was partially restricted, inducing placental hypoxia and ischemia, there was increased expression of preproendothelin mRNA, with the associated proteinuria, renal injury and endothelial dysfunction (Alexander *et al.*, 2001; Makris *et al.*, 2007). It has also become evident that hypertension, due to loss of angiogenic imbalance is associated with increased production of ET-1, leading to the development of PE (George and Granger, 2011; Saleh *et al.*, 2016).

In this study, the AUC of sNGAL was 0.76 with a sensitivity of 46.2 % and specificity, 97.8 % for the screening of PE. The observed sensitivity was quite low compared to previous studies by Artunc-Ulkumen *et al.* (2015) and Rosario D'Anna *et al.* (2010), although the specificity was better or comparable. The AUC of ET-1 was 0.82 and had a sensitivity of 66.8% and a specificity of 92.5% for the prediction of PE. No significant differences were observed between the AUC of sNGAL and ET-1 in PE screening. However, ET-1 could differentiate PS from PNS with an AUC of 0.81, sensitivity, 85.7% and specificity, 82.1% at a cut-off value of 84.0 pg/mL.

The current study has some strengths: this study is among the few studies coming from Ghana that has simultaneously predicted the onset and severity of PE using sNGAL and ET-1. Also, the controls and cases were age-matched and without significant differences in gestational age. The reporting of this study has complied with the guidelines for The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) as much as possible (Von Elm *et al.*, 2014). However, the authors acknowledge some limitations: serum samples were collected after the clinical manifestation and diagnosis of PE. It is recommended to consider sampling

from the first through to the third trimester of pregnancy and after delivery to determine exactly when changes in levels of sNGAL and ET-1 begin to occur (R D'anna *et al.*, 2009).

CONCLUSION

We conclude that there are changes in serum levels of sNGAL and ET-1 in PE and its severe forms. We recommend the inclusion of ET-1 in PE screening panels for the detection and management of PE.

COMPETING INTEREST

Authors declare that they have no competing interests

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Authors' contributions

M. Banyeh and S.J. Adusu conceived the idea and designed the study. S.J. Adusu, S.O. Shittu and W. Tashie performed the experiment and collected the data. M. Banyeh analysed the data and wrote the first draft. All the authors gave critical feedback, read the final draft and approved its content.

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