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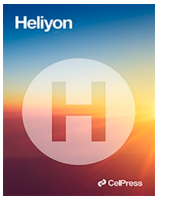

Evaluating circulating soluble markers of endothelial dysfunction and risk factors associated with PE: A multicentre longitudinal case control study in northern Ghana

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ABSTRACT

Serpin E1/PAI-1, N-terminal pro-brain natriuretic peptide (NTpro-BNP) and neuropilin-1 are markers which have been associated with endothelial dysfunction. However, data on the levels of these markers in PE is limited. The limited data on the pathophysiology of PE in relation to these markers necessitated the study.

This was a multicentre case-control study conducted at the Obstetrics and Gynaecology Department of the Tamale Teaching Hospital, the Bawku Presbyterian Hospital and the Bolgatanga Regional Hospital. Out of 520 consenting pregnant women, 127 pregnant women met the inclusion criteria (53 with PE and 74 controls) and were included in this study. Venous, placental, cord and peripheral blood were collected for biomarker assay, haematological parameters and placental parasite determination. Placental tissue sections were obtained for placental malaria and histopathological lesions associated with hypoperfusion. Maternal heart rate and foetal

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umbilical artery Doppler impedance indices; resistance index (RI) and systolic diastolic (SD) ratio were determined to confirm utero-placental hypoperfusion.

Significantly higher proportions of foeto-maternal complications; eclampsia, low birth weight (LBW), neonatal intensive care unit admissions (NICU), intrauterine growth restriction (IUGR), caesarian deliveries and early gestational age at delivery were associated with PE. Women with PE had lower concentrations of platelet ($p = 0.02$) whereas red cell distribution width (RDW) was markedly elevated ($p = 0.01$). NTPro-BNP concentration was markedly elevated ($p = 0.01$) in women with PE whereas neuropilin-1 concentration was lower ($p = 0.03$) compared to the non-PE group. Maternal heart rate was elevated in women with PE and Doppler resistance indices (RI and SD) were significantly elevated in foetuses of PE women than foetuses of the controls. Placental mal-perfusion lesions were higher in women with PE compared to the non-PE group.

Women with PE had increased risk of adverse foeto-maternal complications, significantly associated with placental mal-perfusion lesions, had reduced platelet concentration and elevated RDW-CV levels. NTPro-BNP, RI and SD are elevated in women with PE whereas neuropilin-1 concentration is reduced. Significant changes in these pathological variables in PE women is indicative of significant derangement in endothelial function culminating in adverse maternal and perinatal outcomes of pregnancy.

1. Background

Preeclampsia (PE) complicates 2–8% of pregnancies worldwide [1]. It usually manifests after 20 weeks of gestation in previously normotensive women on at least two occasions more than 4 h apart [1]. The etiology of PE is still unknown as complications usually resolve completely six weeks postpartum. The prevalence of PE in Ghana is estimated to be between 6.55 and 7.03% and is among the five leading causes of maternal and neonatal deaths in the country [2]. In PE, placental barrier thickness, perivillous fibrinoid deposits and syncytial knotting inhibit exchange of oxygen and nutrients between mother and foetus. Additionally, the role of mediators such as NP-1, Serpin E1/PAI-1 and NTpro-BNP have been suggested to regulate endothelial dysfunction [3,4].

Neuropilin-1 (NP-1) activates VEGF by acting as a co-receptor for VEGF165 as it binds to its receptors forming a bridge between VEGF and VEGF-R1/VEGFR2 [5]. Similar to VEGF, NP-1 is upregulated by ischaemia and hypothesized to be expressed in human decidua (functional endometrial layer), syncytiotrophoblast invading trophoblast, endothelial cells of villous capillaries and large vessels during pregnancy but the exact mechanism underpinning its relation with utero-placental hypo-perfusion in PE is still not clear

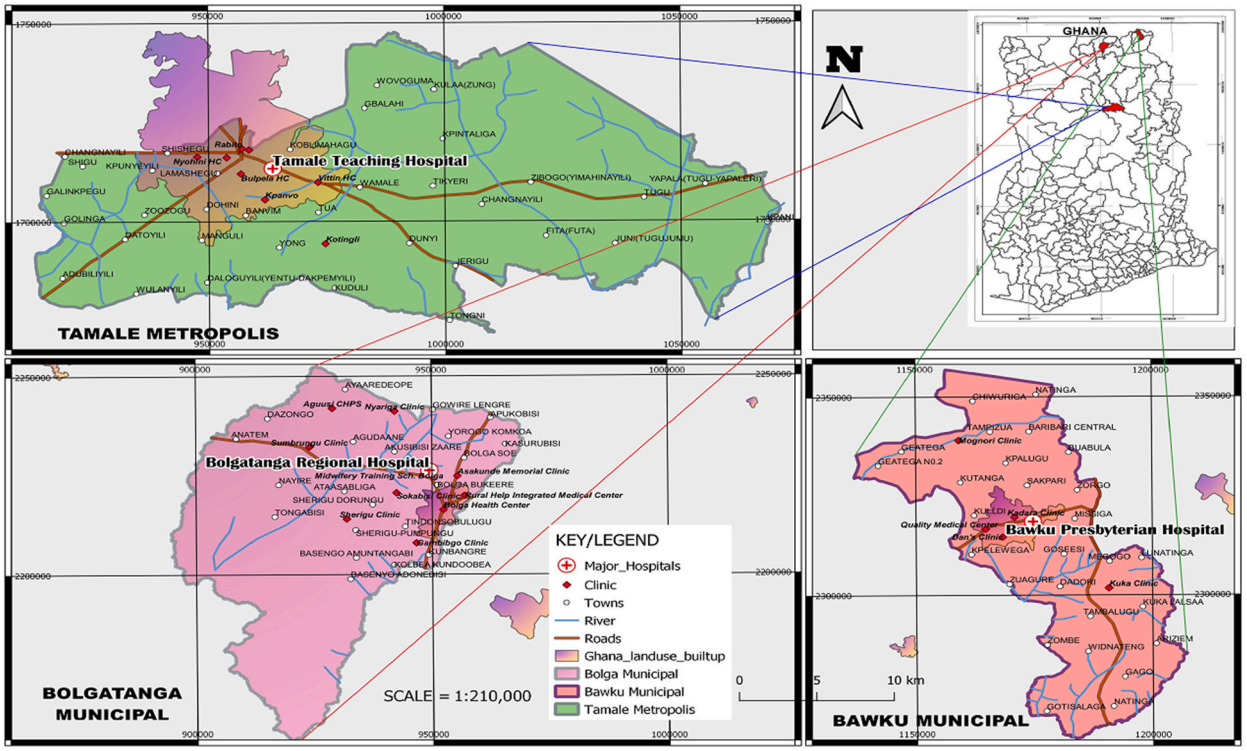

Fig. 1. District map of Ghana showing the three hospitals [10].

[5]. Furthermore, NT-proBNP is a hormone produced by cardiomyocytes in the heart ventricles in response to ventricular stress rather than actual damage or dysfunction of the myocardium [6]. The relation between levels of NTpro-BNP and pregnancy-induced hypertension such as PE remains equivocal as in one breath it has been hypothesized that levels of NT-proBNP increases with increasing severity of PE whereas other studies differ in findings [4,7]. In order to rule out potential causes of cardiovascular compromise associated with PE, ascertaining the relation between circulating levels of NT-proBNP and PE may aid in the early diagnosis and management of the condition.

A limited number of studies have evaluated *P. falciparum* parasitaemia in placental and peripheral blood specimen of PE women. In Ghana, Obiri et al. and her colleagues found higher *P. falciparum* parasitaemia in PE placentas culminating in higher number of placental pathological lesions associated with hypoperfusion such as infarcts, syncytial knots, fibrin deposits, atherosis and calcifications [8]. Elsewhere, a secondary data analysis by Harrington et al. in Thailand found higher *P. falciparum* malaria parasitaemia in peripheral blood samples to be associated with gestational hypertension and PE [9]. Therefore, it will be expedient to evaluate the levels of the biomarkers in relation to *P. falciparum* malaria positivity in multiple sites; placental, cord and peripheral blood in addition to placental tissue parasitaemia, in order to ascertain the sites where these parasites dominate in relation to the markers in severe PE.

Foetal wellbeing is usually evaluated using uterine and umbilical artery Doppler in addition to a 4 hourly foetal heart rate assessment. Despite all these interventions, the pathophysiology of PE is still not properly understood. The Northern part of Ghana lacks basic techniques and equipment for diagnostic testing of PE. It is in light of this that our study sought to evaluate circulating soluble markers of endothelial dysfunction and risk factors associated with PE in Northern Ghana.

2. Methods

2.1. Study area

Three major tertiary referral hospitals in northern Ghana participated in the study. They include the Bawku Presbyterian Hospital (BPH) in Bawku (Upper East Region), the Tamale Teaching Hospital (TTH) in Tamale (Northern Region) and the Bolgatanga Regional Hospital (RHB) in Bolgatanga (Upper East Region). A map of the three study sites is shown in Fig. 1.

2.2. Study design and patient population

The study was a multicenter longitudinal case-control study conducted from February 2019 to April 2020. Participants enrolled were voluntary primigravid pregnant women aged 15–45 years reporting for routine antenatal care. Using the formula described by Charan and Biswas for case control study design with quantitative variables as exposures, the expected sample size was based on assumed expected mean difference between case and control group, 0.60 unit and an assumed standard deviation of 1 unit [11].

$$\text{Sample size (n)} = \frac{(r+1)}{r}\,\frac{SD^2\left(Z_\beta + Z_{\frac{\alpha}{2}}\right)^2}{d^2}$$

r = Ratio of cases to control which is equal to 1 based on equal number of cases and controls selected.
SD = Standard deviation (assumed) = 1 unit.
D = Expected mean difference between case and control (assumed) = 0.60 unit.
Z_β = Standard normal variate for power which is = 80% or 0.84.
$Z_{\alpha/2}$ = Standard normal variate for level of significance. Thus, α = 5% = 1.96.

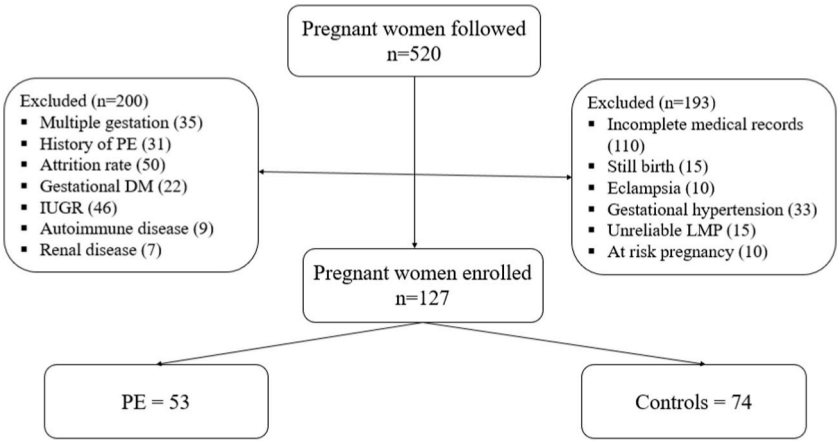

Fig. 2. Exclusion variables and sample size justification used in participant selection.

$$n = \frac{(1+1)}{1} \frac{1^2(0.84+1.96)^2}{0.60^2}$$

$$n = 43.56$$

Approximately, 44 samples were expected to be selected for control group and case group. Therefore, 520 pregnant women who were without PE were recruited and followed until delivery, after written informed consent was obtained from each participant. Participant selection was based on the inclusion criteria of having singleton gestation, attending first antenatal care and of ages ranging from 15 to 45 years. Three hundred and ninety-three (393) out of the 520 pregnant women recruited were excluded based on exclusion variables; being too sick and weak to be subjected to blood sampling, lost to follow-up, eclampsia, receiving emergency care or having an at-risk pregnancy, unable to give informed consent or unwilling to comply with the requirements of the study, women with history of congenital anomaly of foetus, multiple gestations and patients with unreliable last menstrual period. Thus, 127 pregnant women were included in the study of which 53 developed PE (cases) and 74 were controls. Higher number of controls were selected to improve statistical power because it was much easier to recruit controls (Fig. 2).

2.3. Inclusion criteria

Subjects included were pregnant women without PE with singleton gestation attending their first antenatal care of ages ranging from 15 to 45 years.

2.4. Exclusion criteria

Pregnant women who were too sick and weak to be subjected to blood sampling and women receiving emergency care or having an at-risk pregnancy were excluded. Pregnant women who were unable to give informed consent or unwilling to comply with the requirements of the protocol were excluded from the study. Also excluded were women with history of congenital anomaly of foetus, multiple gestations and patients with unreliable LMP (last menstrual period) (Fig. 2).

2.5. Obstetric characteristics and perinatal outcomes

A well-structured closed-ended questionnaire conforming to WHO guidelines [12] was used. The obstetric characteristics evaluated comprised maternal age, gestational age at delivery and parity. Delivery characteristics recorded were mode of delivery, maternal blood pressure at booking, before delivery and after delivery. Adverse maternal outcomes evaluated were labour induction, SCD crisis, preterm delivery (PTD <37 weeks) and eclampsia. Finally, perinatal outcome indicators associated with PE assessed were APGAR score dichotomized into <7 or >7 at 1 min and 5 min, sex of newborn, birth weight, neonatal death, NICU admissions, IUGR and respiratory distress.

2.6. Questionnaire administration

Socio-demographic, antenatal and delivery information, obstetric characteristics and perinatal outcomes were obtained by questionnaire-based interview administered by trained interviewers (midwives) and review of hospital records. Time points for data collection were: at inclusion, prior to delivery, and 48 h postpartum.

2.7. Outcome diagnosis

PE was diagnosed by an Obstetrician Gynaecologist specialist based on a systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg on two separate occasions with proteinuria ($\geq$300 mg/24 h or ++ on a dipstick) after the 20th week of gestation using the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria [13].

2.8. Control selection

The control group was selected by an Obstetrician Gynaecologist specialist based on a systolic blood pressure $<$140 mmHg and/or diastolic blood pressure $<$90 mmHg without proteinuria at time of enrolment after the 20th week of gestation using the ISSHP criteria [13].

2.9. Blood pressure measurement

By utilizing a mercury sphygmomanometer and a stethoscope, trained midwives measured the systolic and diastolic blood pressure of participants. Measurements were taken from the left upper arm after the subjects had been sitting for over 5 min. Two separate readings were recorded at 20-min intervals and the average was considered the participant's blood pressure.

2.10. Anthropometric characteristics

By using WHO standard guidelines, an electronic scale and a meter rule were used to measure body weight and height [14]. BMI was calculated by dividing weight (kg) by height squared (m^2). MUAC is the circumference of the left upper arm and is measured at the mid-point between the tips of the shoulder (acromion process) and elbow (olecranon process).

2.11. Specimen collection and transport

About 5 mls of peripheral blood samples were collected by venipuncture from all participants at 28–32 weeks of gestation. About 2 mls were dispensed into EDTA tubes. Fifty microlitres (50 μl) each of the placental blood, cord blood and peripheral blood was used for *P. falciparum* rapid diagnostic test (RDT) and thick blood film for malaria microscopy, respectively. An additional 50 μl of the blood of each sample type were blotted onto dried Whatman Grade 3 filter papers and stored at −20 °C awaiting DNA extraction and *P. falciparum* species identification by nested polymerase chain reaction assay. The remaining blood was used for haematological tests such as blood grouping, sickling, haemoglobin electrophoresis, full blood count and quantitative G6PD test. About 3 mls of blood was dispensed into gel separator tubes, centrifuged and serum stored at −20 °C at the department of Immunology, Noguchi Memorial Institute for Medical Research (NMIMR) and later used for Human Neuropilin-1, NTpro-BNP and Human Serpin E1/PAI-1 biomarker assay. Placental tissues from PE women and controls were sampled at delivery. A portion of excised tissue (3 cm^3) was washed in physiological saline to remove excess blood and immediately placed in a well labeled specimen container containing 10% neutral buffered formalin for tissue fixation and the avoidance of formalin pigment formation. The fixed placental tissues were transported to the Pathology Department of the Korle-Bu Teaching Hospital (KBTH) for histopathological examination within 72 h of specimen collection.

2.12. Enzyme-linked immunosorbent assay (ELISA)

Serum levels of Human Neuropilin-1 (DY3870-05), NTpro-BNP (DY3604-05) and Human Serpin E1/PAI-1 (DY1786) were assayed using Sandwich ELISA technology according to the manufacturer's instructions (R&D systems, Minneapolis, USA).

2.13. P. falciparum RDT procedure

P. falciparum HRP2 specific malaria RDT was used to diagnose malaria by blotting approximately 5 μL of blood on Ag Pf/Care-Start™ RDT kit (Access Bio., U.S.A.), according to the manufacturer's instructions.

2.14. Malaria parasite identification and quantification by microscopy

Thick blood films were prepared and stained using 10% Giemsa for 15 min. A slide was considered positive if at least one asexual blood-stage *P. falciparum* parasite was identified. *P. falciparum* parasite quantification was done using the thick smear preparation. A thick smear was considered negative for *P. falciparum* if no parasites were observed in 200 high-powered fields. Each blood smear was independently examined by two certified microscopists who were blinded to each other's results. Discrepancies greater than 10% were resolved by a third microscopist.

2.15. Histological sample collection and processing

Placental tissue biopsies obtained after delivery from an off-center region of the placenta approximately halfway between the place of insertion of the umbilical cord and the edge of the placenta were embedded in paraffin. Sections were made using standard techniques and 5 μm thick sections were stained with Haematoxylin-Eosin (H&E) and Giemsa. A Leica ICC50E light microscope equipped with digital microscope camera was used to capture images of the placentas. Some of the parameters were evaluated and analyzed using Leica Las EZ imaging software.

2.16. Slide examination

Histological examinations were performed by two independent pathologists at TTH, blinded to the obstetric and clinical history of the samples. Cases that proved contradictory between observers were re-evaluated until a consensus was reached.

2.17. Histopathological classification of placental malaria

Infection state was classified into four groups namely acute infection (presence of parasites and absence of haemozoin), chronic infection (presence of parasites and significant amount of haemozoin in fibrin or macrophages), past infection (absence of parasites with presence of haemozoin in fibrin or macrophages) and no infection (absence of both parasites and haemozoin in fibrin or macrophages) [15]. Unique pathologies observed during the examination of the placenta were also reported.

2.18. DNA extraction

Plasmodium falciparum parasite DNA was extracted from filter paper dried blood spots using the Tween-20/Chelex extraction method as previously described [16]. For parasite cell lysis, each filter paper dried blood spot (DBS) was punched into a sterile 1.5 ml microfuge tube containing 1 ml 1× phosphate buffered saline (PBS) at a pH of 7.4 supplemented with Tween-20. The tubes were then incubated at room temperature overnight. The reddish supernatant was aspirated and the DBS punches were washed in ice cold 1× PBS and incubated at 4 °C for 30 min. Finally, the supernatant was discarded and 50 μl of freshly prepared 20% Chelex-100 in distilled water and 100 μl of distilled water was added to each tube. The tubes were then incubated at 95 °C for 10 min vortexing at 2 min interval to extract the DNA from the filter paper. The tubes were finally centrifuged at 14000 rpm for 8 min and 120 μl of the supernatant transferred into a new sterile labeled 0.5 ml microfuge tube. Afterwards, the DNA was used immediately or stored at −20 °C.

2.19. Plasmodium falciparum species identification

Nested PCR (limit of detection: 1–10 parasites/μL) was used to amplify unique regions of the *P. falciparum* 18s rRNA gene [17], from genomic DNA extracted from the samples using a previously described protocol [18] with little modifications. In the primary reaction, 10 mM of the genus specific primers rPLU1 (forward) and rPLU5 (reverse) were used in a total reaction volume of 10 μl made up of 2 μl DNA template, 1× PCR buffer, 10 mM dNTPs, 2.5 mM $MgCl_2$ and 1U of OneTaq DNA polymerase. In the secondary reaction (nest 2), the species-specific primers rFAL1/rFAL2 (10 mM) was used in a 1 μl reaction for the identification of *P. falciparum*. The template for the secondary reaction was 0.5 μl of the primary reaction product. The primary and nested PCR reaction cycling conditions included an initial denaturation at 94 °C for 2 min followed by a second denaturation at 94 °C for 30 s, primer annealing step at 55 °C (for primary) and 58 °C (for nested) for 30 s and sequence extension at 68 °C for 1 min 40 s (for primary) and 68 °C for 30 s (for nested) and a final extension at 68 °C for 5 min and 35 cycles. The secondary PCR products were resolved on a 2% agarose gel pre-stained with ethidium bromide, which were subsequently visualized using the UV settings on a Vilbar gel documentation system.

2.20. Radiological scans

Maternal blood pressure was measured before a 12-lead standard ECG recorded and evaluated maternal heart rate, sinus rhythm, sinus tachycardia, sinus bradycardia, axis, PQRST, ventricular hypertrophy and thyrotoxicosis according to the Minnesota code. Using the previous predefined criteria, each ECG was classified as either normal or abnormal.

2.21. Foetal umbilical artery Doppler

Umbilical artery flow velocity consisting of umbilical arterial systolic/diastolic velocity ratio (SDR) and resistance index (RI) were recorded. For all of the ultrasound examinations, a 3.5-MHz sector ultrasound transducer connected to a Toshiba Xario (Toshiba Xario 200, Russia) ultrasound scanner was used. Patients underwent foetal umbilical artery colour Doppler examination at 32–36 weeks of gestation.

2.22. Statistical analysis

Statistical Software Package for Social Scientist version 27.0.1, Graphpad Prism 9 and the Stata Statistical Software version 17 were used to perform the statistical analysis. Categorical data were presented as frequencies (n) and percentages (%). To determine potential factors associated with PE, a univariate logistic regression analysis was performed. This was followed by a multivariate logistic regression analysis using the enter method for variables with p-values$<$0.05 after univariate analysis to identify independent risk factors. For continuous data, normality was checked using Shapiro-Wilk's test, as well as visual inspection with Q-Q plots. Normally distributed data was presented as mean ± SD and significance of difference of biomarkers were evaluated between the presence and absence of condition, using independent *t*-test. Nonparametric data were presented as median (interquartile ranges) and significance of differences of markers were evaluated using Mann-Whitney U tests, where applicable. Receiver operating characteristics curves were computed at an estimated prevalence of PE, 7.03% and confidence interval (CI) of 95% using MedCalc statistical software version 19.8. All tests were two-sided and p-value$<$0.05 was considered statistically significant.

2.23. Ethics approval and consent to participate

Ethical approval for this study was obtained from the institutional review board of the Navrongo Health Research Centre of the Ghana Health Service with approval number NHRCIRB326. Written informed consent was obtained from all participants who opted to participate after the aims and objectives of the study had been explained to them. Participants consented to have their anonymous ultrasound images published. Consent for minors were obtained from the parents/guardians. Participation was voluntary, and respondents were assured that the information obtained was strictly for research and academic purposes only and were guaranteed the liberty to opt out from the study at their own convenience.

3. Results

Antenatal and delivery information from 2016 to 2020 is shown in Table S2.

3.1. Socio-demographic and obstetric characteristics of study participants

The average ages of the PE and control cohort were similar (28.43 (SD: 7.33) vs. 27.11 (SD: 5.97), p = 0.29). Participants with PE presented with a higher 1st trimester BMI (26.10 vs. 23.70 kg/m^2, p = 0.03), a higher MUAC (26.30 vs. 24.30 cm, p=<0.01), early deliveries (37.50 vs. 39.00 weeks, p=<0.01), had more caesarian deliveries (65.79 vs. 14.89%, p=<0.01) and were associated with eclampsia (9.43 vs. 0.00%, p = 0.01) compared to the control group (**Table 1**).

3.2. Haemodynamics and haematological parameters of participants

Systolic and diastolic blood pressure readings at study inclusion, at delivery and 48 h post-delivery were consistently elevated in women with PE compared to the control group. Furthermore, a higher proportion (7.5 vs. 2.7%, p = 0.23), of the participants with PE

Table 1
Socio-demographic and obstetric characteristics of subjects stratified according to PE.

Maternal characteristics	Non-PE (n = 74)	PE (n = 53)	p-values
Demographic			
Maternal age (years)*: Mean ± SD	27.11 ± 5.97	28.43 ± 7.33	0.29
			0.24
15–19	8 (11.43)	4 (8.51)	
20–29	40 (57.14)	20 (42.55)	
30–34	13 (18.57)	11 (23.40)	
35–45	9 (12.86)	12 (25.53)	
Marital status			0.45
Married	71 (95.95)	49 (92.45)	
Single	3 (4.05)	4 (7.55)	
Occupation			0.87
Civil servant	18 (24.32)	11 (20.75)	
Self-employed	32 (43.24)	25 (47.17)	
Unemployed	24 (32.43)	17 (32.08)	
Clinical variables: Median (IQR)			
BMI at 1st trimester (kg/m^2)	23.70 (20.50–26.50)	26.10 (21.25–31.60)	**0.03**
MUAC (cm)	24.30 (22.10–27.20)	26.30 (24.55–27.95)	<**0.01**
Gestational age at delivery (weeks)	39.00 (38.00–40.00)	37.50 (35.00–39.00)	<**0.01**
Gravidity*			0.85
Unigravida	26 (36.11)	20 (37.74)	
Multigravida	46 (63.89)	33 (62.26)	
Parity*			0.42
Nulliparity	29 (40.28)	21 (39.62)	
Uniparity	17 (23.61)	8 (15.09)	
Multiparity	26 (36.11)	24 (45.28)	
Mode of delivery*			<**0.01**
Vaginal delivery	40 (85.11)	13 (34.21)	
Caesarian delivery	7 (14.89)	25 (65.79)	
Labor induced*			0.14
No	47 (95.92)	35 (85.37)	
Yes	2 (4.08)	6 (14.63)	
Eclampsia			**0.01**
No	74 (100.00)	48 (90.57)	
Yes	0 (0.00)	5 (9.43)	
Peripheral blood *P. falciparum* infection (PCR)*			0.48
No	30 (68.18)	21 (58.33)	
Yes	14 (31.82)	15 (41.67)	
Placental blood *P. falciparum* infection (PCR)*			0.24
Negative	26 (100.00)	23 (88.46)	
Positive	0 (0.00)	3 (11.54)	
Cord blood *P. falciparum* (PCR)*			1.00
Negative	25 (86.21)	24 (82.76)	
Positive	4 (13.79)	5 (17.24)	

Categorical variables are presented as frequency with percentage in parenthesis. MUAC: mid-upper arm circumference, BMI: body mass index, PE: preeclampsia, non-PE: non-preeclamptic, IQR: interquartile range. *: Some values in cases and controls did not sum up due to missing data. Maternal age: Non-PE (4) & PE (6), gravidity: Non-PE (2), parity: Non-PE (2), mode of delivery: Non-PE (27) & PE (15), labour induced: Non-PE (25) & PE (12), peripheral blood *P. falciparum* infection (PCR): Non-PE (30) & PE (17), placental blood (PCR): Non-PE (48) & PE (27), Cord blood (PCR): Non-PE (45) & PE (24).

were sickling positive compared to non-PE group (Table 2).

3.3. Haematological parameters of participants

Although most haematological parameters were similar between the two groups, women with PE had lower platelet concentration (155.00 vs. 201.50 $\times$ 10^3/μL, p = 0.02) and higher RDW-CV (19.40 vs. 13.40%, p = 0.01) compared to the non-PE group (**Table 3**).

3.4. Neonatal characteristics and adverse outcomes

Participants with PE delivered neonates who were lower in weight compared to non-PE women (2.80 vs. 3.00, p = 0.01). Majority of the neonates born to women with PE had intrauterine growth restriction (20.00 vs. 2.04%, p = 0.01), and were admitted to the neonatal intensive care unit (24.39 vs. 6.12%, p = 0.02) (**Table 4**).

3.5. Comparing malaria positivity by RDT, microscopy and PCR in placental, cord and peripheral blood specimen

The nested polymerase chain reaction (PCR) was the main procedure used to confirm *P. falciparum* infection state in cord, placental and peripheral blood specimen of study participants. It detected more positive cases of *P. falciparum* DNA than microscopy and rapid diagnostic test (RDT) for malaria in the PE group compared to the non-PE group (**Tables S3 and S4**).

3.6. Levels of soluble markers of endothelial dysfunction between PE and Non-PE groups

Whereas NT-proBNP was elevated (p = 0.01), neuropilin-1 was lower (p = 0.03) in the PE group than the Non-PE. Additionally, Serpin E1/PAI-1 was marginally elevated in women who developed PE compared to the Non-PE group (Fig. 3A).

3.7. Levels of radiological imaging parameters stratified according to PE status

Radiological imaging showed an elevation (p < 0.05) in umbilical artery Doppler parameters including higher resistance index (RI), systolic diastolic (SD) ratio and maternal heart rate among women who developed PE compared to the Non-PE group (Fig. 3B).

Table 2
Haemodynamic and haematological parameters of participants stratified according to PE.

Variable	Non-PE (n = 74)	PE (n = 53)	p-value
Sickling			0.23
Negative	72 (97.30)	49 (92.45)	
Positive	2 (2.70)	4 (7.55)	
Hb genotype			0.24
AA	71 (95.95)	49 (92.45)	
AS	3 (4.05)	2 (3.77)	
SC	0 (0.00)	2 (3.77)	
SS	0 (0.00)	0 (0.00)	
Blood group			0.29
A negative	6 (8.11)	3 (5.66)	
A positive	15 (20.27)	9 (16.98)	
AB positive	8 (10.81)	5 (9.43)	
B negative	3 (4.05)	1 (1.89)	
B positive	10 (13.51)	16 (30.19)	
O negative	3 (4.05)	0 (0.00)	
O positive	29 (39.19)	19 (35.85)	
G6PD deficiency			0.17
No defect	73 (98.65)	51 (96.23)	
Full defect	1 (1.35)	0 (0.00)	
partial defect	0 (0.00)	2 (3.77)	
Blood pressure measurements (mmHg): Median (IQR)			
SBP 1st visit	104.50 (100.00–111.00)	120.00 (109.00–133.50)	<**0.01**
DBP 1st visit	60.00 (60.00–70.00)	78.00 (64.00–87.50)	<**0.01**
SBP at delivery	116.00 (110.00–129.00)	151.00 (142.00–157.00)	<**0.01**
DBP at delivery	71.00 (67.00–80.00)	96.00 (87.00–102.00)	<**0.01**
SBP 48 h postpartum	110.00 (102.00–120.00)	130.00 (123.00–149.00)	<**0.01**
DBP 48 h postpartum	68.12 ± 1.59	85.61 ± 2.43	<**0.01**

Categorical variables are presented as frequency with percentage in parenthesis. SBP: Systolic blood pressure, DBP: diastolic blood pressure, PE: preeclampsia, non-PE: non-preeclamptic, G6PD: glucose-6-phosphate dehydrogenase, Hb: haemoglobin.

Table 3
Haematological parameters of participants stratified according to PE.

Variables	Non-PE (n = 74)	PE (n = 53)	p-values
RBC ($x10^6/\mu L$)	3.90 (3.61–4.43)	3.98 (3.64–4.65)	0.76
Hb (g/dL)	10.90 (10.00–11.60)	10.50 (9.70–11.80)	0.65
HCT (%)	34.80 (32.26–39.70)	34.70 (32.10–39.56)	0.99
MCV (fL)	88.10 (78.80–93.30)	87.10 (79.00–94.00)	0.94
MCH (pg)	26.90 (24.50–29.30)	26.70 (24.20–28.60)	0.31
MCHC (g/dL))	31.90 (28.90–34.0)	30.00 (28.20–32.30)	0.19
RDW-CV (%)	13.40 (12.10–18.20)	19.40 (13.00–23.40)	**0.01**
RDW-SD (fL)	50.10 (42.65–71.60)	60.15 (48.80–78.50)	0.11
WBC ($x10^3/\mu L$)	7.08 ± 2.90	7.12 ± 3.49	0.95
Neutrophil (%)	63.09 (46.10–75.70)	62.00 (38.10–73.56)	0.72
Lymphocyte (%)	27.80 (18.30–43.46)	21.81 (14.60–34.4)	0.26
PLT ($x10^3/\mu L$)	201.50 (166.50–293.50)	155.00 (128.00–238.00)	**0.02**

RBC: red blood cell, Hb: haemoglobin, HCT: haematocrit, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, RDW-CV: red cell distribution width-correlation variance, RDW-SD: red cell distribution width-standard deviation, WBC: white blood cell, PLT: platelet, PE: preeclampsia, Non-PE: non-preeclamptic. *P*-value <0.05 is considered statistically significant different.

Table 4
Neonatal characteristics and adverse outcomes.

Neonatal characteristics	Non-PE (n = 74)	PE (n = 53)	p-value
Baby sex*			0.40
Female	25 (51.02)	17 (41.46)	
Male	24 (48.98)	24 (58.54)	
Neonatal anthropometrics:			
Median (IQR)			
Birth weight (kg)	3.00 (2.80–3.20)	2.80 (2.10–3.00)	**0.01**
Baby length (cm)	50 (48–52)	49 (44–51)	0.08
Head circumference (cm)	33 (32–34)	33 (31–35)	0.87
Chest circumference (cm)	32 (29–34)	32 (29–33)	0.52
APGAR at 1 min*			0.75
<7	5 (10.42)	6 (14.63)	
>7	43 (89.58)	35 (85.37)	
APGAR at 5 min*			0.24
<7	2 (4.17)	5 (12.20)	
>7	46 (95.83)	36 (87.80)	
NICU admission*			**0.02**
No	46 (93.88)	31 (75.61)	
Yes	3 (6.12)	10 (24.39)	
Respiratory distress*			0.70
No	46 (93.88)	37 (90.24)	
Yes	3 (6.12)	4 (9.76)	
Neonatal death*			0.59
No	48 (97.96)	39 (95.12)	
Yes	1 (2.04)	2 (4.88)	
IUGR*			**0.01**
No	48 (97.96)	32 (80.00)	
Yes	1 (2.04)	8 (20.00)	

Categorical variables are presented as frequency with percentage in parenthesis. PE: preeclampsia, Non-PE: non-preeclamptic. APGAR: appearance, pulse, grimace, activity and respiration, NICU: neonatal intensive care unit, IUGR: intrauterine growth restriction, BMI: body mass index, MUAC: mid-upper-arm circumference, Hb: haemoglobin. *P*-value <0.05 is considered statistically significant different. *: Some values in cases and controls did not sum up due to missing data. Baby sex: Non-PE (25) & PE (12), APGAR at 1 min: Non-PE (26) & PE (12), APGAR at 5 min: Non-PE (26) & PE (12), NICU admission: Non-PE (25) & PE (12), respiratory distress: Non-PE (25) & PE (12), neonatal death: Non-PE (25) & PE (12), IUGR: Non-PE (25) & PE (13).

3.8. Crude and adjusted odds ratio of biomarkers associated with PE

A unit increase in the concentration of NT-proBNP (COR = 1.06, 95% CI (1.01–1.10), p = 0.01) was significantly associated with increased odds of PE whereas a unit increase in the concentration of neuripilin-1 (COR = 0.99, 95% CI (0.97–0.99), p = 0.04) was significantly associated with reduced odds of PE. In a multivariate analysis, both markers were independently associated with PE **(Table 5).**

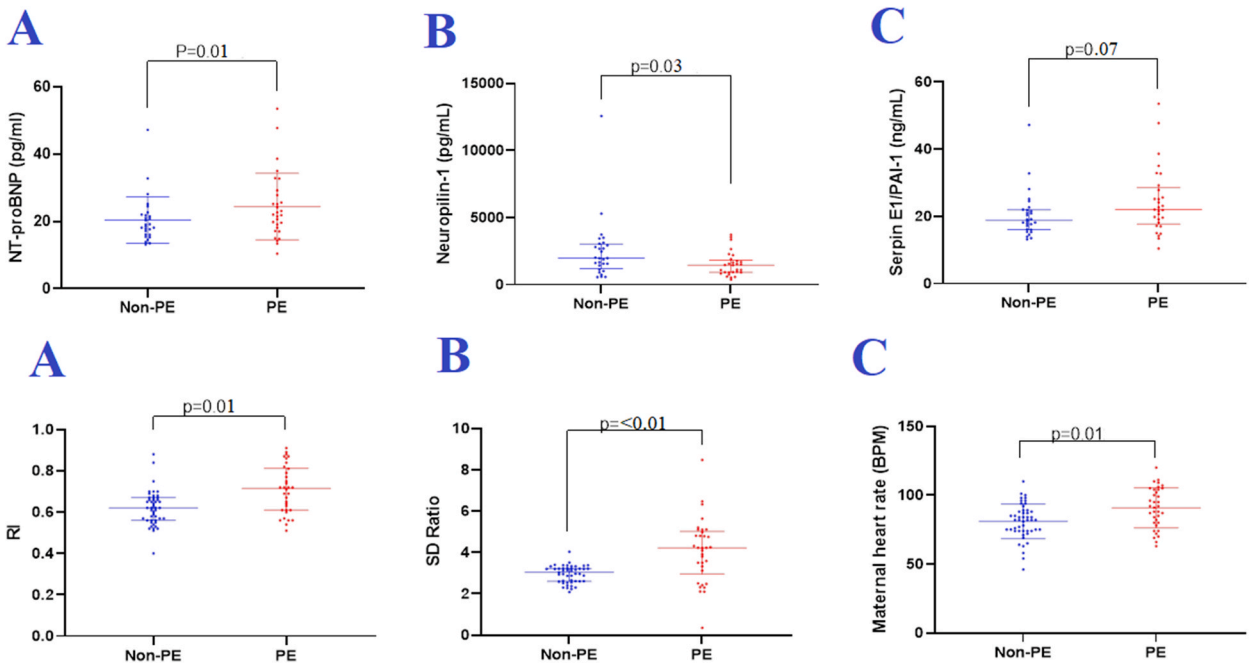

Fig. 3A. Levels of soluble markers of endothelial dysfunction between PE and Non-PE groups. (A) Levels of NT-proBNP between PE and Non-PE. (B) Levels of neuropilin-1 between PE and Non-PE. (C) Levels of serpin E1/PAI-1 between PE and Non-PE. NT-proBNP: N-terminal pro-brain natriuretic peptide, PE: preeclampsia, Non-PE: non-preeclamptic. *P*-value <0.05 is considered statistically significant different.
Fig. 3BLevels of radiological imaging parameters stratified according to PE status. (A) Levels of RI between PE and Non-PE. (B) Levels of SD ratio between PE and Non-PE. (C) Levels of maternal heart rate between PE and Non-PE. RI: resistance index, SD: systolic diastolic, PE: preeclampsia, Non-PE: non-preeclamptic, BPM: beats per minute. *P*-value <0.05 is considered statistically significant different.

Table 5
Crude and adjusted odds ratio of biomarkers associated with PE.

Variable	COR (95% CI)	*P*-values	AOR (95% CI)	*P*-values
NT-proBNP(pg/ml)	1.06 (1.01–1.10)	**0.01**	1.09 (1.03–1.16)	<**0.01**
Neuropilin-1(pg/ml)	0.99 (0.97–0.99)	**0.04**	0.99 (0.97–0.99)	**0.01**
Serpin E1/PAI-1(ng/mL)	1.06 (0.98–1.14)	0.10		

NT-proBNP: N-terminal pro-brain natriuretic peptide, COR: crude odds ratio, AOR: adjusted odds ratio. *P*-value <0.05 is considered statistically significant different.

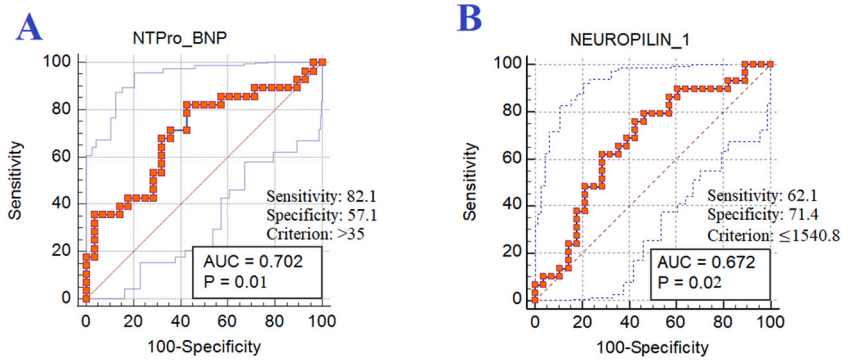

Fig. 4A. Diagnostic performance of soluble markers of endothelial dysfunction in predicting PE beyond the 20th week of gestation. (A) N-terminal pro-brain natriuretic peptide (B) Neuropilin-1.

3.9. Diagnostic performance of soluble markers of endothelial dysfunction in predicting PE beyond the 20th week of gestation

In predicting PE, NT-ProBNP had an excellent sensitivity (82.1 vs. 62.1%), poor specificity (57.1 vs. 71.4%) and a good area under the curve (AUC) (0.702 vs. 0.672) compared to neuropilin-1 (Fig. 4A).

3.10. Diagnostic performance of RI, SD ratio and maternal heart rate in predicting PE beyond the 20th week of gestation

The radiological marker, SD ratio, had the highest (good) sensitivity (70.6 vs. 52.9%), excellent specificity (95.7 vs. 93.6%) and a good AUC (0.761 vs. 0.722) compared to RI. Maternal heart rate had the lowest sensitivity (52.8%), specificity (79.6%) and AUC (0.686), of the three markers (Fig. 4B & 4C).

Fig. 5 Shows normal and abnormal umbilical artery Doppler velocity waveform patterns of 4 pregnant women at term.

3.11. Histopathological lesions potentially associated with PE

Pregnant women with PE were significantly associated with placental morphologies such as syncytial knots (9.00 vs. 6.00%, p = 0.04), infarction (48.57 vs. 16.22%, p = 0.01), sickled RBCs (60.00 vs. 5.41%), p=<0.01), hemorrhage in intervillous spaces (82.86 vs. 13.51%), p=<0.01), cytotrophoblastic proliferation (82.86 vs. 45.95%), p = 0.<01), small sclerotic villi (60.00 vs. 0.00%), p=<0.01), hypermatured villi (82.86 vs. 40.54%), p=<0.01), villous fibrosis (97.14 vs. 48.65%, p=<0.01) and perivillous hyalination (74.29 vs. 29.73%, p=<0.01). **(Table 6).**

Fig. 6A and B shows placental pathological lesions associated with PE.

4. Discussion

4.1. Obstetric and adverse pregnancy outcomes

Evidence suggests that obesity is associated with increased risk of PE [19,20]. Consistently, in this study, participants with PE had higher BMI and MUAC compared to controls. Similar to the findings of our study, Endeshaw et al. in Ethiopia, found increasing MUAC (≥25 cm = obese) to be consistent with increasing odds of PE [21]. Additionally, Motedayen et al. in Iran found an association between hypertension and high BMI in pregnancy [22]. Indeed, MUAC is considered a better choice of gestational weight gain measurement compared to BMI because changes in MUAC have been found to be minimal during the entire course of gestation [23].

The women who developed PE in our cohort delivered earlier than those who did not have PE. In addition, the mode of delivery was by caesarian section and most of these neonates were later admitted to the neonatal intensive care unit. Some pregnant women with PE also developed eclampsia. This finding concurs well with the findings of Dassah et al. in Ghana who found PE to be closely associated with eclampsia which resulted in a high risk of caesarian sections and preterm deliveries [24]. Usually, mode of delivery for pregnant women with PE is by obstetric indications. Nonetheless, if there is evidence of foetal compromise at a gestational age remote from term, women with PE may deliver by caesarian section [25]; otherwise, delivery will be delayed expectantly to attain full foetal growth and maturation in-utero to limit foetal complications such as prematurity, microsomia, intrauterine growth retardation, intrauterine foetal demise and LBW [26].

Maternal systolic and diastolic blood pressures at ANC booking, prior to delivery and 48 h post-delivery were consistently elevated in the women who developed PE than the control group. PE is diagnosed with an elevation in blood pressure at or beyond the 20th week of gestation with proteinuria [13]; hence 1st trimester elevation in systolic and diastolic blood pressure is indicative of hypertensive disorders of pregnancy which is predictive of PE at the 20th week of gestation and beyond [27].

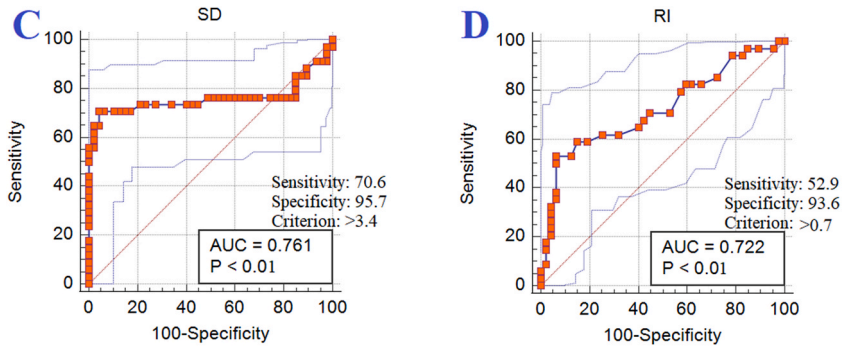

Fig. 4B. Diagnostic performance of RI and SD ratio in predicting PE beyond the 20th week of gestation. (C) SD ratio: systolic diastolic ratio, (D) RI: resistance index.

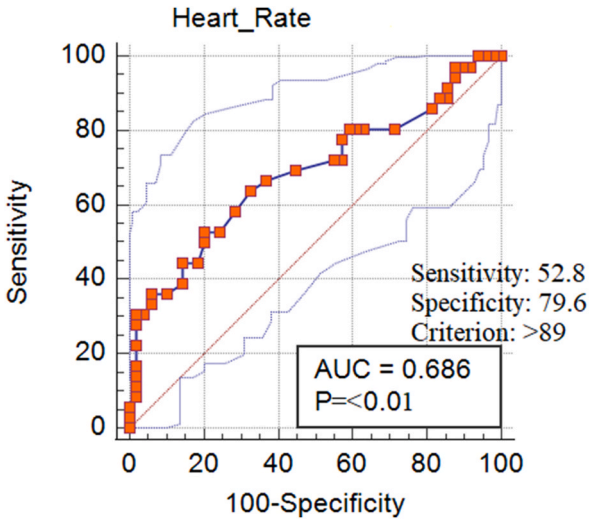

Fig. 4C. Diagnostic performance of maternal heart rate in predicting PE beyond the 20th week of gestation. (E) Maternal heart rate.

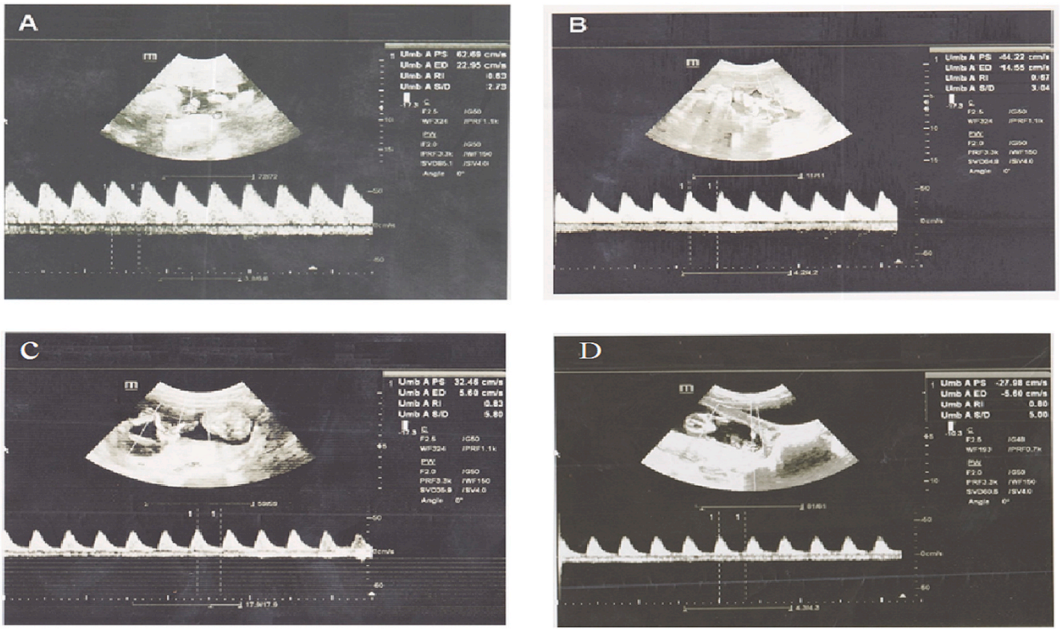

Fig. 5. Shows umbilical artery Doppler velocity waveform patterns of 4 pregnant women at term. A and B show normal umbilical artery Doppler (normal vessel) pattern, C and D show reduced end-diastolic flow.

4.2. Levels of circulating markers of endothelial dysfunction in PE and normotensive controls

Data regarding the association between NT-proBNP and neuropilin-1, and PE is limited. A significantly higher serum concentration of NT-proBNP and lower neuropilin-1 concentrations were found in the PE group compared to the control group. Furthermore, a unit increase in NT-proBNP concentration was associated with a higher likelihood of developing PE whereas a unit increase in neuropilin-1 concentration was associated with a reduced likelihood of developing PE. NT-ProBNP had an excellent sensitivity and a good area under the curve compared to neuropilin-1. However, neuropilin-1 had a good specificity than NT-ProBNP. Although data on the role of NT-proBNP in reproductive and placental physiology is wanting, the findings of this study concur with the findings of Borghi et al. in Italy, who found significantly higher levels of serum NT-proBNP in third trimester PE women compared to healthy normotensive controls [28]. A number of reports have been made about NT-proBNP concentrations in placental circulation, amniotic fluid and increased NT-proBNP expression in syncytiotrophoblasts [29,30].

The higher likelihood of developing PE due to a marked elevation in NT-proBNP concentration beyond the 20th week of gestation

Table 6
Histopathological lesions potentially associated with PE.

Variables	Non-PE (n = 74)	PE (n = 53)	p-values
Placental morphology			
Syncytial knotting (%)	6.00 (5.00–10.00)	9.00 (4.00–15.00)	**0.04**
Syncytial rapture (%)	8.00 (4.00–10.00)	10.00 (6.00–12.00)	0.28
Necrosis area*			
Negative	31 (83.78)	26 (74.29)	
Positive	6 (16.22)	9 (25.71)	0.39
WBCs in placental villi			
Total leukocytes	16.50 (7.00–34.00)	22.00 (9.00–46.00)	0.43
Mononuclear cells	9.00 (4.00–15.00)	6.00 (4.00–14.00)	0.59
Polymorphonucleates	5.50 (0.00–20.00)	8.50 (2.00–20.00)	0.26
Infarction*			
Negative	31 (83.78)	18 (51.43)	
Positive	6 (16.22)	17 (48.57)	**0.01**
Sickled cells*			
Negative	35 (94.59)	14 (40.00)	
Positive	2 (5.41)	21 (60.00)	<**0.01**
Calcification*			
Negative	29 (78.38)	23 (65.71)	
Positive	8 (21.62)	12 (34.29)	0.30
Hemorrhage in intravillous space*			
Negative	32 (86.49)	6 (17.14)	
Positive	5 (13.51)	29 (82.86)	<**0.01**
Hyalinized villi	8.50 (5.00–15.00)	10.00 (6.00–15.00)	0.45
Fibrinoid necrosis*			
Negative	27 (72.97)	27 (77.14)	
Positive	10 (27.03)	8 (22.86)	0.79
Cytotrophoblastic proliferation*			
Negative	20 (54.05)	6 (17.14)	
Positive	17 (45.95)	29 (82.86)	<**0.01**
Accelerated villous maturation	10.00 (6.00–19.00)	10.00 (5.00–15.00)	0.70
Small sclerotic villi*			
Negative	37 (100.00)	14 (40.00)	
Positive	0 (0.00)	21 (60.00)	<**0.01**
Decidual vasculopathy*			
Negative	34 (91.89)	28 (80.00)	
Positive	3 (8.11)	7 (20.00)	0.18
Hypermatured villi*			
Negative	22 (59.46)	6 (17.14)	
Positive	15 (40.54)	29 (82.86)	<**0.01**
Villous fibrosis*			
Negative	19 (51.35)	1 (2.86)	
Positive	18 (48.65)	34 (97.14)	<**0.01**
Perivillous hyalination*			
Negative	26 (70.27)	9 (25.71)	
Positive	11 (29.73)	26 (74.29)	<**0.01**
P. falciparum parasite count	23.24 ± 46.85	32.59 ± 52.35	0.45
Haemozoin*			
Negative	14 (41.18)	9 (28.13)	
Positive	20 (58.82)	23 (71.88)	0.31

Categorical variables are presented as frequency with percentage in parenthesis. Values are presented as MUAC: mid-upper arm circumference, PE: preeclampsia, Non-PE: non-preeclamptic. *P*-value <0.05 is considered statistically significant different. *: Some values in cases and controls did not sum up due to missing data. Non-PE (37) & PE (18) were missing for variables; infarction, necrosis area, sickle cell, calcification, hemorrhage in intervillous space, villous fibrosis, perivillous hyalination, hypermatured villi, decidual vasculopathy, small sclerotic villi, cytotrophoblastic proliferation, fibrinoid necrosis. However, Non-PE (40) & PE (21) were missing for haemozoin. Refer to Table S1 for units of parameters and parasite scores.

together with an elevation in maternal heart rate seen in the PE group of this study culminated in a significant derangement in endothelial function and uteroplacental hypoperfusion. This finding concurs well with the findings of Iacovidou et al. in Turkey who discovered that elevated serum NT-proBNP concentration is proportional to the degree of left ventricular diastolic dysfunction [31]. This might have resulted in the haemodynamic instability of our PE cohort culminating in higher number of placental malperfusion lesions and umbilical artery resistance seen in this study.

The concentration of NT-proBNP and abnormal umbilical artery Doppler parameters suggestive of underperfusion were significantly higher in the PE group compared to the control group. This finding is in variance with the findings of Uyar et al. in Turkey who investigated the association between abnormal uterine artery Doppler velocity waveforms and NT-proBNP concentration in the second trimester to predict PE. They did not find any significant changes in the concentrations of NT-proBNP in both groups in the second trimester. This implies that the concentrations of NT-proBNP might peak at delivery (third trimester) confirming PE pathogenesis (onset) at second trimester, and severe forms of the disease at third trimester evidenced by the high number of placental vascular and

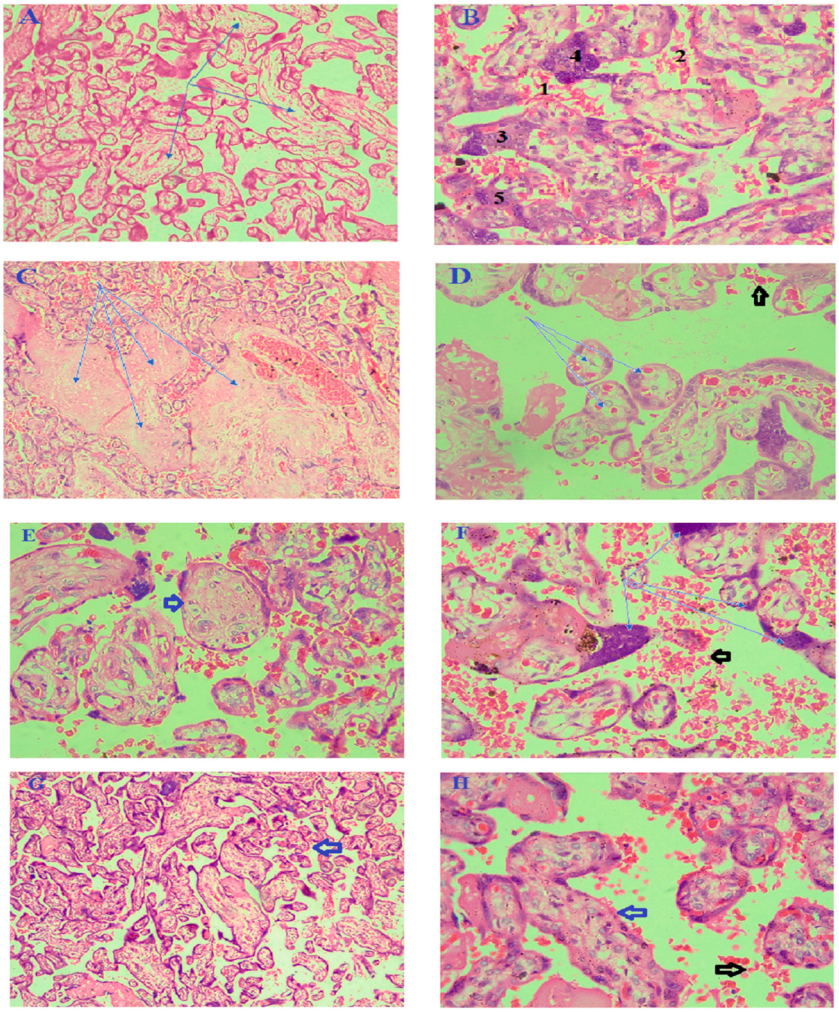

Fig. 6A. Shows placental histological lesions (A) Placenta showing normal villi viewed under ×10. (B) 1 and 2 show placental sickling and 3–5 show cytotrophoblastic proliferation viewed under ×10. (C) Infarction viewed under ×10 objective (D) Small sclerotic villi is represented by blue arrows and black arrows represent sickled RBCs viewed under ×40.
Fig. 6B: Shows placental histopathological lesions. (E) Villous fibrosis observed under ×10 objective (F) Blue arrows represents syncytial knots whereas the black arrow represents intervillous space hemorrhage made up of normal RBCs and sickled RBCs viewed under ×10 magnification (G) Hypermatured villi viewed under ×40 objective (H) Shows perivillous hyalination represented by blue arrow and intervillous space hemorrhage represented by black arrow viewed under ×40 objective.

malperfusion lesions seen in PE placentas at delivery in this study.

The lower serum concentrations of neuropilin-1 reported in this study correlates with deficient placentation and inadequate remodelling of spiral arteries resulting in limited uteroplacental perfusion (hypoxia). In humans, hypoxia can lead to upregulation of placental neuropilin-1 and VEGF gene in a bid to increase angiogenesis and uteroplacental perfusion [32]. The consequences of PE are enormous as it causes defective placentation, placental ischaemia, and endothelial cell dysfunction. Neuropilin-1 is a transmembrane protein which cannot induce cellular responses independently because it is without a tyrosine kinase domain. It acts as co-receptor for vascular endothelial growth factor (VEGF), forming a bridge between VEGF and VEGF receptors (VEGF-R1/VEGFR2), to signal placental angiogenesis. VEGF is an important key player in angiogenesis and vascular remodelling during the implantation process. Apart from VEGFR1 and VEGFR2, which are the two most established receptors for VEGF signaling, neuropilin-1 has also been shown to play an important role in VEGF signaling hence promoting angiogenesis in human endometrium during the menstrual cycle as it acts as an additional co-receptor for VEGF [5]. Consequently, an increasing concentration of neuropilin-1 resulted in a reduced likelihood of developing PE in our cohort.

It is noteworthy that, adequate blood supply is required at the early stages of pregnancy for successful implantation of the blastocyst. That is why in the first trimester, the formation and invasion of new blood vessels is one of the most important steps for efficient implantation, whereas in the latter part of gestation, the growing embryo is supplied exclusively by the placenta [33]. This finding serves as a significant milestone in elucidating uteroplacental circulation as it opens several doors of speculations into the actual

pathophysiology of neuropilin-1. It has therefore been hypothesized that neuropilin-1 may play a principal role in the vascular remodelling process during embryonic development and the establishment of a pregnancy [34]. Furthermore, since there have been very few studies on this important angiogenic marker in PE, data on the expression levels of this marker in the trophoblast, syncytiotrophoblast and amniotic fluid in women with PE is still wanting. There is also paucity of data on serum concentrations of this marker in PE women and normotensive controls. This limitation prompted this comparative study to determine serum concentrations of this marker in women with PE and normotensive controls. The low serum concentration of neuropilin-1 seen in women with PE correlates with the findings of Arad et al. in Israel, who by seeking to investigate the expression of neuropilin-1 in human placentas, found lower expression of neuropilin-1 in the syncitiotrophoblast of PE placentas compared to the controls [3].

4.3. Changes in haematological parameters in women with PE at term

Red cell distribution width-coefficient of variation (RDW-CV) was significantly elevated in women with PE compared to normotensive controls. Elevated RDW-CV which is a measure of anisocytosis is a haematological parameter for definitive diagnosis of iron deficiency anaemia. Recently, elevation in RDW-CV has been associated with hypertensions and cardiovascular diseases but the exact mechanism is yet to be elucidated [35]. In determining the diagnostic value of RDW-CV, Shachan et al. in India found RDW to have sensitivity of 85.3% for mild PE and 93.8% for severe PE. Furthermore, RDW had a specificity of 49% for mild PE and 44.1% for severe PE [36]. High systemic RDW-CV in hypertensions is speculated to be a marker of inflammation [16]. Nevertheless, our study is limited as we could not include novel biomarkers of inflammatory response such as C-reactive protein, heat shock protein 70 (hsp 70), erythrocyte sedimentation rate (ESR), cytokines and interleukins to strongly ascertain the finding; further studies are warranted.

In resource-limited settings, RDW, platelet and mixed (eosinophil, basophil and monocytes) are inexpensive markers (component of full blood count) that can be used as diagnostic and prognostic markers for PE [37,38]. Low platelet count and increased cytotrophoblastic proliferation was associated with PE in this study. Cytotrophoblasts deposition in spiral arteries, apoptosis and increased syncytiotrophoblast apoptosis result in fibrin deposition and platelet activation [39]. Furthermore, increased platelet destruction may be due to immune factors, hyper-platelet activation, increased platelet consumption due to excessive bleeding or endothelial dysfunction and platelet aggregation [40]. Redman et al. [41] related endothelial activation to inflammatory responses such as intravascular leukocytosis, platelet aggregation and complement system activation. In pregnancy, bone marrow disorders or nutritional factors can also lead to low platelet concentration [42].

4.4. Umbilical artery Doppler velocimetry and heart rate of PE women

To further confirm placental insufficiency based on the increase in placental lesions and significant changes in circulating markers of endothelial dysfunction observed in our study, we relied on umbilical artery Doppler impedance indices RI and SD. Maternal heart rate was also measured by ECG. There was a significant increase in umbilical artery Doppler impedance indices (RI and SD) and maternal heart rate in the PE group compared to the control group. Elevation in maternal heart rate is not surprising as it confirms consistent elevation in maternal haemodynamics (systolic and diastolic blood pressures) at booking, prior to delivery and 48 h post-delivery as previously discussed. Of note, maternal echocardiographic findings at 24 weeks of gestation reveal that patients who subsequently develop early severe maternal and foetal complications through elevations in maternal haemodynamics and elevated maternal total vascular resistance is consistent with cardiovascular mediated placental disorder as seen in PE and intrauterine growth restriction (IUGR) [43]. The high velocity waveform due to elevations in resistance indices RI and SD ratio found in PE concurs with abnormally high resistance flow velocity in uterine vasculature. This may be as a result of failure of cytotrophoblastic cells to invade myometrial segments of placental spiral artery bed to maintain its musculoelastic arterial structure evidenced by significant elevations in placental lesions as seen in this study [44].

There is dearth of data on screening characteristics of umbilical artery Doppler resistance indices RI and SD ratio in predicting PE. Thus, the predictive capacity of these indicators was assessed. ROC curve analysis for RI yielded a poor sensitivity but an excellent specificity with a good area under the curve in predicting PE. On the other hand, SD ratio had a good sensitivity and an excellent specificity with a good area under the curve in predicting PE. A second trimester uterine artery Doppler study by Bodova et al. [45] in Slovakia found moderate area under the curve for RI and SD in predicting PE. Furthermore, maternal heart rate had a poor sensitivity, a good specificity and a moderate area under the curve in predicting PE. On the contrary, Tejera et al. in Portugal found excellent sensitivity and specificity of maternal heart rate in predicting PE [46]. The conflicting findings highlights the impact of disparate demographics on the performance of diagnostic indicators and warrants further region-specific studies.

4.5. Placental pathological changes associated with PE

Maternal vascular (placental sickling and hemorrhage in intravillous spaces) and placental mal-perfusion lesions (placental infarction, syncytial knotting, stromal fibrosis, cytotrophoblast proliferation, small sclerotic villi, hypermatured villi and perivillous hyalination) were significantly associated with women with PE. Studies regarding placental pathological changes is limited in our study area. A meta-analysis by Oteng-Ntim et al. in the UK found sickle cell disease to be associated with PE [47]. The etiology of PE is linked to pathological changes in the placenta as removal of the placenta results in a decline in symptoms [48]. PE is characterized by defective placentation due to inadequate remodelling of the spiral arteries in the placental bed due to superficial ingrowth of trophoblasts in the decidua [49]. This leads to hypoperfusion, hypoxia, and oxidative stress. In later stages, it is also characterized by the clinical manifestations of hypertension, proteinuria and edema as a result of maternal endothelial damage and systemic inflammation

[50]. The actual mechanism regarding the association between placental malperfusion lesions and PE is not clear. We also found higher cases of LBW and IUGR among participants with PE. This may be as a result of suboptimal placental function due to decreased oxygen and nutrient delivery to the developing foetus.

4.6. Strength and weakness of the study

Our study derives its strength from the combination of placental pathological findings, soluble markers of endothelial dysfunction, umbilical artery Doppler resistance indices RI and SD, haematological parameters and adverse foeto-maternal complications all in one cohort since previous studies looked at these variables individually. Our study however is limited by the lack of more markers such as eNOS-Trafficking inducer (NOSTRIN), asymmetric dimethylarginine (*ADMA*), platelet endothelial cell adhesion molecule-1 (PECAM-1), E-selectin and *P*-selectin predictive of endothelial and organ damage such as kidney and liver function. These are worth considering in future studies.

5. Conclusion

Women with PE have increased risk of adverse foeto-maternal complications, significant placental malperfusion lesions, elevated platelet and RDW-CV levels. NTPro-BNP and umbilical artery Doppler resistance indices (RI and SD) are elevated in women with PE whereas neuropilin-1 concentration is reduced. Significant changes in these pathological variables in the PE women is indicative of significant derangement in endothelial function culminating in adverse maternal and perinatal outcomes of pregnancy. Women should be educated on the foeto-maternal complications (dangers) posed by PE to enhance their strict adherence to routine antenatal care to aid in early detection and management of the condition. Due to the high cost of various diagnostic tests for PE, we entreat Government, policy holders, philanthropists, non-governmental agencies and religious groups to intervene in making these diagnostic technologies available in low resourced settings.

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Author contribution statement

Benjamin Ahenkorah, Ben Gyan, Samuel Asamoah Sakyi, Linda Ahenkorah Fondjo, Gideon Helegbe, Winfred Ofosu and Eddie Owiredu Williams: Conceived and designed the experiments; Wrote the paper.

Benjamin Ahenkorah and Edmund Muonir Der: Performed the experiments.

Benjamin Ahenkorah and Eddie Owiredu Williams: Analyzed and interpreted the data.

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Data availability statement

Data associated with this study has been deposited at https://doi.org/10.6084/m9.figshare.15130371.v1.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19096.

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