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Maternal serum angiopoietins levels in pre-eclampsia and pregnancy outcomes

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Abstract

Background and Aims: Although the understanding of pre-eclampsia (PE) has improved, there is still insufficient knowledge on the exact etiology and pathophysiological mechanisms. Dysregulation of angiogenic factors has emerged as a significant contributing factor. Among these factors, angiopoietins (Ang-1 and Ang-2) have gained considerable attention due to their crucial role in regulating vascular development and endothelial function. This study explored the maternal serum levels of angiopoietins and perinatal outcomes in PE.

Methods: A case-control study involving women with PE (cases) and normotensive pregnancies (controls) was conducted at the Maternity unit of the Korle-Bu Teaching Hospital. Descriptive analysis was performed and the Mann–Whitney U test (two-sided) was used to compare maternal serum levels of angiopoietins between the cases and controls.

Results: We included 188 participants comprising 94 cases (women with PE) and 94 controls (normotensive pregnancies) with an average maternal age of 29.76 ± 5.56 and 28.43 ± 5.57 years, respectively. Maternal serum levels of Ang-2 were significantly lower among the PE cases compared to the normotensive controls (1.25 [0.90, 2.15] vs. 2.14 [1.18, 5.73] ng/mL, $p = 0.001$) but no significant difference in Ang-1 levels (92.61 [80.92, 114.92] vs. 99.26 [81.76, 113.12] ng/mL, $p = 0.429$) was observed between the groups. The Ang-1/Ang-2 ratio was significantly elevated among women with PE compared to normotensive controls (74.47 [37.69, 110.59] vs. 45.98 [16.11, 88.22] ng/mL, $p = 0.014$). Also, women who delivered vaginally had significantly high maternal serum levels of Ang-1 compared to women who had cesarean section delivery (107.98 ± 27.79 vs. 89.02 ± 32.62 ng/mL).

Conclusion: Maternal serum levels of Ang-2 but not Ang-1 were significantly depressed in women with PE compared to the pregnant normotensive controls. No significant associations were observed between Ang-1, Ang-2 levels, or the Ang-1/Ang-2 ratio and pregnancy outcomes such as preterm birth, birth weight, and severity of hypertension.

KEYWORDS

angiopoietins, maternal serum levels, pre-eclampsia, pregnancy outcome, normotensives

1 | INTRODUCTION

Pre-eclampsia (PE) is the most prevalent hypertensive disorder in pregnancy, marked by new onset hypertension and proteinuria after 20 weeks of gestation and resulting in multi-organ dysfunction including the renal, hepatic, cardiac, and brain.[1] PE is frequently associated with adverse maternal and perinatal outcomes, particularly in low- and middle-income countries (LMICs). The global incidence of PE ranges from 3% to 8% of all pregnancies and varies significantly across countries.[2] In Ghana, hypertensive disorders in pregnancy including PE account for approximately 18% of maternal deaths[3] compared to the global figure of about 15%.[4]

Although the understanding on PE has improved, there is still insufficient knowledge concerning the exact etiology and pathophysiological mechanisms.[5,6] The updated hypothesized pathophysiological mechanism of PE relates to abnormal placentation and maternal systemic disease leading to systemic endothelial dysfunction. However, the specific mechanism by which this abnormal placentation leads to systemic endothelial dysfunction has remained elusive despite extensive clinical and biological investigations.[7] Evidence suggests a crucial imbalance in the pathogenesis of PE, primarily involving antiangiogenic and proangiogenic factors. Key among these are antiangiogenic factors like soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), as well as proangiogenic factors such as vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF). This imbalance plays a crucial role in the pathogenesis of PE resulting in inadequate vasculogenesis and angiogenesis in the placenta, and placental hypoperfusion and ischemia.[5,8] SFlt-1 hinders the interaction between angiogenic factors such as VEGF and PlGF with their respective receptors,[9] inhibiting their ability to support the stability of endothelial cells, resulting in compromised angiogenesis and reduced production of vasodilators like nitric oxide and prostacyclin.[10] Also, elevated levels of SFlt-1 may be associated with reduced levels of angiopoietins, especially angiopoietin 1 (Ang-1), contributing to the characteristic vascular dysfunction and hypertension associated with PE. Consequently, it prompts an increased expression of hypoxia-inducible transcription factors and vasoactive factors, such as sFlt-1 and sEng, surpassing the placental expression of VEGF. This imbalance also results in widespread dysfunction of vascular endothelial cells.

In the investigational attempt to further understand the pathophysiology of PE, angiopoietins (Ang-1 and Ang-2) have been implicated in the pathogenesis, especially in vascular morphogenesis, remodeling and development of placental vasculature.[8,11,12] During early pregnancy, the placenta plays a crucial role in expressing Ang-1, Ang-2, and Tie-2 receptors which are crucial to the development of placental vasculature.[8] This expression is essential for the establishment and maintenance of proper vasculature, ensuring adequate blood supply to support fetal growth and development. Disruptions can therefore lead to compromised maternal–fetal exchange and development of PE. Angiopoietins notably bind to the same endothelial cell-specific receptor tyrosine kinase (Tie-2). This binding is regulated by the presence of VEGF which causes the decoupling and downregulation of Tie-2. Although the mechanistic pathways of these proangiogenic factors, typically expressed by the placenta, differ significantly, they complement the action of VEGF.[8,13] For instance, Ang-1 plays a protective role in normal pregnancy by promoting endothelial cell maturation, integrity, and vascular stability. Throughout gestation, the levels of Ang-1 are normally elevated. Ang-1 also reduces vascular permeability induced by VEGF. On the other hand, Ang-2 causes endothelial cell destabilization, increased vascular permeability and apoptosis in the absence of VEGF, potentially having detrimental effects during normal pregnancy. However, in the presence of VEGF, Ang-2 renders endothelial cells accessible to VEGF's effects.[8,13]

Other studies have shown that women with PE exhibit increased levels of Ang-1 in an attempt to counteract the vascular dysfunction associated with PE.[11,14] Conversely, decreased serum levels of Ang-2, an antagonist of Ang-1, contributes to vascular destabilization observed in PE.[15,16]

According to Apostolakis et al.,[17] Ang-1/Ang-2 ratio showed a sensitivity of 47% and specificity of 87% in 25–28 weeks pregnant women who subsequently developed PE and can be used as a predictive biological marker for the diagnosis of late-onset PE. Although the role of angiopoietins in PE has been described in other studies, findings on their association with adverse maternal and perinatal outcomes have been conflicting and not adequately studied, especially in LMICs.[18] The study findings emphasize the significance of angiopoietins' imbalance in the pathophysiology of PE and highlight the potential capacity of these molecules as diagnostic markers and therapeutic targets in managing PE.

Given the burden of PE and limited research in Ghana, coupled with their potential contribution to the pathophysiology, the association between angiopoietins and pregnancy outcomes in PE highlights further understanding on its clinical management. This study explores the maternal serum levels of angiopoietins (Ang-1 and Ang-2) in women with PE compared to normotensive pregnancies and the associations between angiopoietins and pregnancy outcomes.

2 | METHODOLOGY

2.1 | Study design and setting

The study was a case-control design conducted at the Department of Obstetrics and Gynaecology, Korle-Bu Teaching Hospital (KBTH), Accra, Ghana from April to June 2011. KBTH is Ghana's largest tertiary healthcare institution, which conducts approximately 11,000 deliveries annually. In the hospital, about one in every five women receiving maternity services present with hypertensive disorders including PE.[19] The study adhered to the REporting Recommendation for Tumor Marker Prognostic Studies (REMAK) checklist[20] following the guidelines for reporting statistics in clinical research related to urology.[21]

2.2 | Study population

The inclusion criteria comprised pregnant normotensive women (controls) and pregnant women with an established diagnosis of PE (cases) between 18 and 42 years. The pregnant women must have had an ultrasound scan during their first trimester for accurate pregnancy dating. The exclusion criteria consisted of pregnant women with a history of chronic medical conditions such as long-term hypertension, diabetes mellitus, kidney disease, thyroid dysfunction, urinary tract infection, inflammatory disorders, and cardiac disease. The sample size for this study was determined using the formula:

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

where SD is standard deviation = 2.21. This was determined using a single population SD from a study by Phupong et al.[18] r is the ratio of cases to control, thus, one for equal number of cases and control. Z_β is the standard normal variate for the power of 90% = 1.28. $Z_{\alpha/2}$ is the standard normal variate for the level of significance at 95% = 1.96. d is the Phupong et al.'s[18] expected mean difference between cases and controls was 1.1.

$$\text{Sample size} = \frac{1+1}{1} + \frac{(2.21)^2(1.28+1.96)^2 = 85}{(1.1)^2}.$$

The minimum sample size for the study calculated based on case/control ratio of 1:1 was 85 which was rounded to 94 participants each for cases and controls. For both cases and controls, an ultrasound scan was done in the first trimester for accurate gestational age estimation.

The American College of Obstetricians and Gynaecologists (ACOG) criteria adopted for the diagnosis of PE include blood pressure of ≥140/90 mmHg measured two consecutive times, at least 4 h apart, and proteinuria (defined as ≥300 mg/24-h urine protein loss or ≥1+ dipstick on a random urine sample) after 20th-week gestation in a previously normotensive woman.[22] Early-onset PE was defined as PE that develops before 34 weeks of gestation, whereas late-onset PE develops at ≥34 weeks of gestation.[23] We defined severe PE as diastolic blood pressure ≥110 mmHg and or systolic blood pressure ≥160 mmHg, and mild PE as diastolic blood pressure <110 mmHg and or systolic blood pressure <160 mmHg.

2.3 | Recruitment and sampling of study participants

In this study, a case-control design was utilized to examine the levels of angiopoietins in the maternal serum of women with PE (cases) compared to those with normotensive pregnancies (controls). The research was conducted at the Maternity unit of the KBTH in Accra, Ghana. The study population included pregnant women aged between 18 and 42 years, with accurate pregnancy dating based on a first-trimester ultrasound scan.

To ensure comparability between the cases and controls, a systematic random sampling technique was employed to recruit study participants from the prenatal clinic and the emergency department of the hospital. Thorough interviews were conducted, and data on demographic and clinical characteristics were collected. Information regarding blood pressure measurements, height, weight, and other relevant variables was extracted from the participants' medical records. The diagnostic criteria for PE were based on the guidelines provided by the ACOG, which included elevated blood pressure readings and the presence of proteinuria after the 20th week of gestation. By following established diagnostic criteria, the study aimed to provide valuable insights into the levels of angiopoietins in PE and their potential association with adverse pregnancy outcomes.

The recruitment process involved approaching eligible participants and obtaining their informed consent before inclusion in the study. Standardized structured data extraction forms were used during the interviews to gather comprehensive information. The diastolic and systolic blood pressures of the participants were measured using a mercury sphygmomanometer while they were in a reclining posture. Additional details such as blood pressure at the initial antenatal care visit, height in meters, maternal age in years, gravidity, and parity were obtained from the participants' medical records. Heights and weights were recorded using the participants' antenatal clinic booklet. Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters.

2.4 | Inclusion criteria

- Pregnant women aged between 18 and 42 years.
- Accurate pregnancy dating based on a first-trimester ultrasound scan.
- Pregnant women with normotensive pregnancies (controls).
- Pregnant women diagnosed with PE (cases).

2.5 | Exclusion criteria

- Pregnant women with chronic medical conditions (e.g., diabetes, hypertension, kidney disease).
- Pregnant women outside the specified age range (below 18 years or above 42 years).
- Pregnant women without accurate pregnancy dating based on a first-trimester ultrasound scan.
- Pregnant women with incomplete or unreliable medical records.

2.6 | Ethical considerations

The Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (protocol number: CHS-Et/M.1–4.9/

2021-2022) provided approval for this study. Further, all potential study participants gave their informed consent before their recruitment into the study. Throughout this study, procedures commensurate with ethical guidelines were followed.

2.7 | Blood sampling and laboratory analysis

After a tourniquet was applied, a sample of about 5 mL of venous blood was taken from the cubital fossae of the study participants. In pregnant women with PE, the blood samples were taken at the time of diagnosis before the start of any antihypertensive medication including magnesium sulfate administration. Within 1 h of collection, the blood samples were centrifuged at 250 rpm for 10 min, and the serum was aliquoted into cryotubes and stored at −80°C till the laboratory assays were done. In this study, enzyme-linked immunosorbent assay test kits (DY923 and DY623; R&D Systems) were used to measure serum Ang-1 and Ang-2 levels (Quantikine). The assays were carried out strictly according to the manufacturers' instructions.

2.8 | Data analysis

The statistical analysis for the present study followed the recommended guidelines outlined by Assel et al.[21] Data analysis was performed using SPSS version22.0 and R statistical package (version 3.6.2, R Core Team, Vienna, Austria). Initial descriptive analysis was performed using descriptive statistics such as mean, median standard deviation, and percentiles. Maternal parameters, including maternal age, parity, gestational age, and blood pressure at recruitment and birth, as well as birth weight, were assessed for normal distribution using the Shapiro–Wilk test. For normally distributed variables, the results were expressed as mean ± standard deviation, and comparisons were made using an independent Student's *t*-test. The equality of variances between groups was tested using Levene's test. Maternal serum angiopoietins levels were not normally distributed based on the Shapiro–Wilk test and were presented as median (interquartile range). Mann–Whitney *U* test (two-sided) was used to compare the serum levels of angiopoietins between the control and the cases, pregnancy outcomes as well as the preeclamptic groups (mild vs. severe PE and early vs. late onset PE). A $p < 0.05$ was considered as statistically significant.

3 | RESULTS AND FINDINGS

We included 188 pregnant women in the study comprising 94 pregnant women with an established diagnosis of PE (cases) and 94 pregnant normotensives (controls). The mean (±SD) maternal ages for the cases and controls were 29.76 ± 5.56 and 28.43 ± 5.57 years, respectively. Women with PE had significantly lower parity compared to pregnant normotensives (0.77 ± 1.23 vs. 1.32 ± 1.34). The gestational age at birth was significantly lower in women with PE compared with normotensive pregnant women (36.20 ± 1.71 vs. 38.91 ± 1.28 weeks). The mean systolic and diastolic blood pressures at recruitment for the PE group (164.97 ± 19.46 and 105.94 ± 10.82 mmHg, respectively) were significantly elevated compared to the pregnant normotensive women (111.71 ± 11.40 and 69.64 ± 8.50 mmHg), respectively ($p < 0.05$). The BMI was relatively higher in cases compared to the controls (29.45 ± 6.26 vs. 27.33 ± 8.72, respectively) but the difference did not reach statistical significance (Table 1).

Further, significant adverse pregnancy outcomes occurred among pregnant women with PE compared to the pregnant normotensives. For instance, PE was significantly associated with lower birth weight compared to the pregnant normotensives (2469.26 ± 696.75 vs. 3237.23 ± 507.63 g). Also, the proportions of poor Apgar scores (<7) at 1 and 5 min were higher in PE compared to the women with normotensive pregnancy. The cesarean section and

TABLE 1 General characteristics, angiopoietin levels and pregnancy outcomes of women with pre-eclampsia and normotensive pregnancy.

Characteristic	Pregnant normotensive (*n* = 94) Mean ± SD	Pre-eclampsia group (*n* = 94) Mean ± SD	*p*-Value
Maternal age (years)	28.43 ± 5.57	29.76 ± 5.56	0.10
Parity	1.32 ± 1.34	0.77 ± 1.23	0.004
GA at recruitment (weeks)	34.88 ± 2.44	34.31 ± 2.74	0.13
SBP at recruitment (mmHg)	111.71 ± 11.40	164.97 ± 19.46	<0.001
DBP at recruitment (mmHg)	69.64 ± 8.50	105.94 ± 10.82	<0.001
Pregnancy outcomes			
GA at birth (weeks)	38.91 ± 1.28	36.20 ± 1.71	<0.001
Birth weight (grams)	3.24 ± 0.51	2.47 ± 0.70	<0.001
Mode of delivery			<0.001
Vaginal	76 (74.5)	26 (25.5)	
Cesarean	18 (20.9)	68 (79.1)	
Preterm birth			
Yes	2 (2.1)	48 (51.1)	<0.001
No	92 (97.9)	46 (48.9)	
Low-birth weight			<0.001
Yes	4 (4.3)	44 (46.8)	
No	90 (95.7)	50 (53.2)	

Note: Statistically significant at $p < 0.05$ on independent Student *t*-test.
Abbreviations: BMI, basal metabolic rate; DBP, diastolic blood pressure; GA, gestational age; SBP, systolic blood pressure; SD, standard deviation.

preterm birth rates were 79.1% ($n = 68$) and 51.1% ($n = 48$), respectively in the PE group compared to normotensive women.

3.1 | Maternal serum concentration of Ang-1, Ang-2, and Ang-1/Ang-2 ratio in PE

Based on systolic blood pressure at diagnosis, mild and severe PE occurred in 37.2% ($n = 35$) and 62.8% ($n = 59$), respectively. Similarly, mild and severe PE occurred in 47.9% ($n = 45$) and 52.1% ($n = 49$), respectively based on diastolic classification. Based on systolic and or diastolic classification, mild and severe PE occurred in 24.5% ($n = 23$) and 75.5% ($n = 71$), respectively. Although serum levels of Ang-1 were reduced in women with PE compared to the pregnant normotensives the difference was not statistically significant (92.61 [80.92, 114.92] vs. 99.26 [81.76, 113.12] ng/mL). Maternal serum Ang-2 levels were significantly lower in women with PE compared to pregnant normotensives (1.25 [0.90, 2.15] vs. 2.14 [1.18, 5.73] ng/mL). Also, Ang-1/Ang-2 ratio was significantly elevated among women with PE than the pregnant controls [74.47 [37.69, 110.59] vs. 45.98 [16.11, 88.22] ng/mL) as shown in Table 2. Further, no significant differences were detected concerning maternal serum Ang-1, Ang-2, and Ang-1/Ang-2 ratio between the mild and severe PE (Table 3).

PE was further categorized into early onset ($n = 29$) and late-onset ($n = 65$). There was no statistical difference between early onset and late onset concerning maternal serum Ang-1 levels (94.04 [64.47, 117.18] vs. 92.28 [81.79, 114.92] ng/mL, $p = 0.81$), Ang-2 levels (1.08 [0.83, 2.80] vs. 1.26 [0.98, 1.94] ng/mL, $p = 0.94$), and Ang-1/Ang-2 levels (58.62 [19.38, 97.38] vs. 76.07 [40.13, 107.33] ng/mL, $p = 0.23$). Figure 1A–F indicates the comparison of normotensive pregnancy versus PE and early onset versus late onset PE in terms of maternal serum levels of angiopoietins. There was no significant difference between the controls and cases in terms of maternal serum levels of Ang-1 ($p = 0.43$). However, Ang-2 levels were significantly reduced in PE compared to normal pregnancy ($p = 0.001$). In addition, the Ang-1/Ang-2 ratio was significantly higher in the PE group compared to normotensive women ($p = 0.014$). There was no statistically significant difference ($p > 0.05$) in angiopoietins levels between early onset and late onset PE (Figure 1A–F).

There was a significant positive correlation between Ang-1 and the whole study population. However, further analysis indicated that the positive correlation was limited to normotensive pregnancy (Figure 2A–C). There was no significant correlation between PE and Ang-1, Ang-2 or Ang-1–Ang-2 ratio. Similarly, there was no correlation between maternal serum angiopoietin levels and early and late onset PE (Figure 2D–F).

Women who delivered vaginally had significantly high maternal serum levels of Ang-1 compared to women who had cesarean section (104.36 [84.97, 131.66] vs. 90.22 [68.53, 109.55] ng/mL) but there were no significant variations in the levels of Ang-2 or Ang-1/Ang-2 ratio (Table 4).

Similarly, there was no significant association between angiopoietin levels and preterm birth (gestational age birth) and low-birth weight ($p > 0.05$). Based on diastolic blood pressure at birth, mild and severe hypertension occurred in 80.9% ($n = 76$) and 19.1% ($n = 18$), respectively. Similarly, mild and severe hypertension at birth occurred in 6.4% ($n = 6$) and 93.6% ($n = 88$), respectively based on systolic blood pressure classification. Classification based on systolic and or diastolic blood pressures at birth resulted in mild and severe PE occurred in 5.3% ($n = 5$) and 94.7% ($n = 89$), respectively. There was no significant difference between the severity of blood pressure at birth and maternal serum angiopoietin levels ($p > 0.05$) (Table 4).

4 | DISCUSSION

4.1 | Summary of key findings

In this study, we assessed maternal serum angiopoietin levels in PE and normotensive pregnancies and their association with pregnancy outcomes. We determined that the maternal serum level of Ang-2 was significantly lower in PE compared to normotensive pregnancy

TABLE 2 Maternal serum concentration of Ang-1, Ang-2, and Ang-1/Ang-2 ratio in pre-eclampsia and normotensive pregnancies.

Maternal biomarker	Pregnant normotensives Median (IQR)	Pre-eclampsia Median (IQR)	*p*-Value
Ang-1 (ng/mL)	99.26 (81.76, 113.12)	92.61 (80.92, 114.92)	0.430
Ang-2 (ng/mL)	2.14 (1.18, 5.73)	1.25 (0.90, 2.15)	0.001
Ang-1/Ang-2	45.98 (16.11, 88.22)	74.47 (37.69, 110.59)	0.014
	Angiopoietins in early- and late-onset PE		
	Early-onset	Late-onset	*p*-Value
Ang-1 (ng/mL)	94.04 (64.47, 117.18)	92.28 (81.79, 114.92)	0.81
Ang-2 (ng/mL)	1.08 (0.83, 2.80)	1.26 (0.98, 1.94)	0.94
Ang-1/Ang-2	58.62 (19.38, 97.38)	76.07 (40.13, 107.33)	0.23

Note: Statistically significant at $p < 0.05$ using Mann–Whitney *U*-test.

Abbreviations: Ang-1, angiopoietin 1; Ang-2, angiopoietin 2; IQR, interquartile range.

TABLE 3 Maternal serum angiopoietin levels and pregnancy outcomes in severe and mild PE.

Biomaker	Classification by systolic BP at diagnosis			Classification by diastolic BP at diagnosis			Classification by systolic and diastolic BP at diahnosis		
	Mild PE	Severe PE	*p*-Value	Mild PE	Severe PE	*p*-Value	Mild PE (n = 71)	Severe PE (N = 117)	*p*-Value
Ang-1 (ng/mL)	91.96 (79.59, 113.48)	93.09 (80.92, 117.18)	0.69	90.72 (80.92, 113.46)	94.04 (79.91, 118.22)	0.83	85.58 (57.20, 109.55)	94.04(81.79, 117.18)	0.28
Ang-2 (ng/mL)	1.25 (0.96, 2.80)	1.28 (0.87, 1.94)	0.52	1.38 (1.01, 2.54)	1.13 (0.84, 1.87)	0.43	1.26 (0.96, 2.88)	1.25 (0.87, 2.04)	0.59
Ang-1/Ang-2 ratio	68.93 (33.42, 127.57)	74.10 (37.62, 104.42)	0.95	64.46 (32.50, 106.99)	74.57 (38.19, 107.33)	0.74	61.62 (21.32, 113.84)	74.47 (37.76, 106.99)	0.56

Abbreviations: Ang-1, angiopoietin 1; Ang-2, angiopoietin 2; BP, blood pressure; PE, pre-eclampsia.

(1.25 [0.90, 2.15] vs. 2.14 [1.18, 5.73 ng/mL). Although the serum level of Ang-1 showed a downward trend in PE compared to normotensive pregnancy (92.61 [80.92, 114.92] vs. 99.26 [81.76, 113.12] ng/mL) the difference was not statistically significant. Also, Ang-1/Ang-2 ratio was significantly elevated among women with PE than the pregnant controls (74.47 [37.69, 110.59] vs. 45.98 [16.11, 88.22] ng/mL). There was a significant positive association between Ang-1 level and gestation age at birth.

4.2 | Interpretation of findings

Ang-1 and Ang-2 are proangiogenic factors that promote endothelial health, vascular maturation, and stability leading to angiogenesis by binding to the same endothelial cell-specific tyrosine kinase receptor within the placental vascular, and their activity is tightly regulated by the physiological action of VEGF.[8,13,24] In normal pregnancy, Ang-1 and Ang-2 exhibit antagonistic properties. For instance, Ang-1 induces vascular cell maturation, growth, and stabilization, whiles Ang-2 causes apoptosis and destabilization of the vessel growth in the absence of VEGF.[11,12,14,24] In our study, Ang-2 levels were significantly reduced in PE when compared to normotensive pregnancy similar to Hirokoshi et al.[15] who determined significantly reduced Ang-2 levels in PE in Japan. This finding suggests that alterations in Ang-2 levels may be associated with the development and pathophysiology of PE. Ang-2 is known for its crucial role in regulating angiogenesis and vascular integrity, and its reduction in PE implies a disruption in normal vascular development and function during pregnancy. The decreased levels of Ang-2 in PE could contribute to the characteristic endothelial dysfunction associated with PE. Endothelial dysfunction is a key feature of PE, leading to impaired blood flow and organ damage. The reduction in Ang-2 may disrupt the delicate balance between angiogenic and antiangiogenic factors, favoring an antiangiogenic state and promoting the development of PE. These findings highlight the potential utility of Ang-2 as a biomarker for PE diagnosis or monitoring. Measuring Ang-2 levels in pregnant women could provide valuable information about their risk of developing PE or the progression of the disease.

On the other hand, Han et al. reported a significant increase in both placental messenger RNA expression of Ang-2 and maternal plasma Ang-2 protein was significantly increased in patients with severe PE.[25] Also, Leinonen et al. reported that Ang-2 levels are significantly raised before clinical diagnosis of PE.[11] In a recent longitudinal study in Thailand, Phupong et al.,[18] determined no significant difference in the maternal angiopoietin levels of women who developed PE and normotensive controls contrary to our study findings.

In our study, there was no significant difference in the serum levels of angiopoietin in terms of severity of PE (mild vs. severe) and onset of the disease (early onset vs. late onset). Similar findings were reported by Hirokoshi et al.[15] remarking no significant difference in Ang-2 levels between mild and severe PE. This finding suggests that serum levels of angiopoietin may not be a reliable marker for predicting the severity or onset of PE. The lack of significant

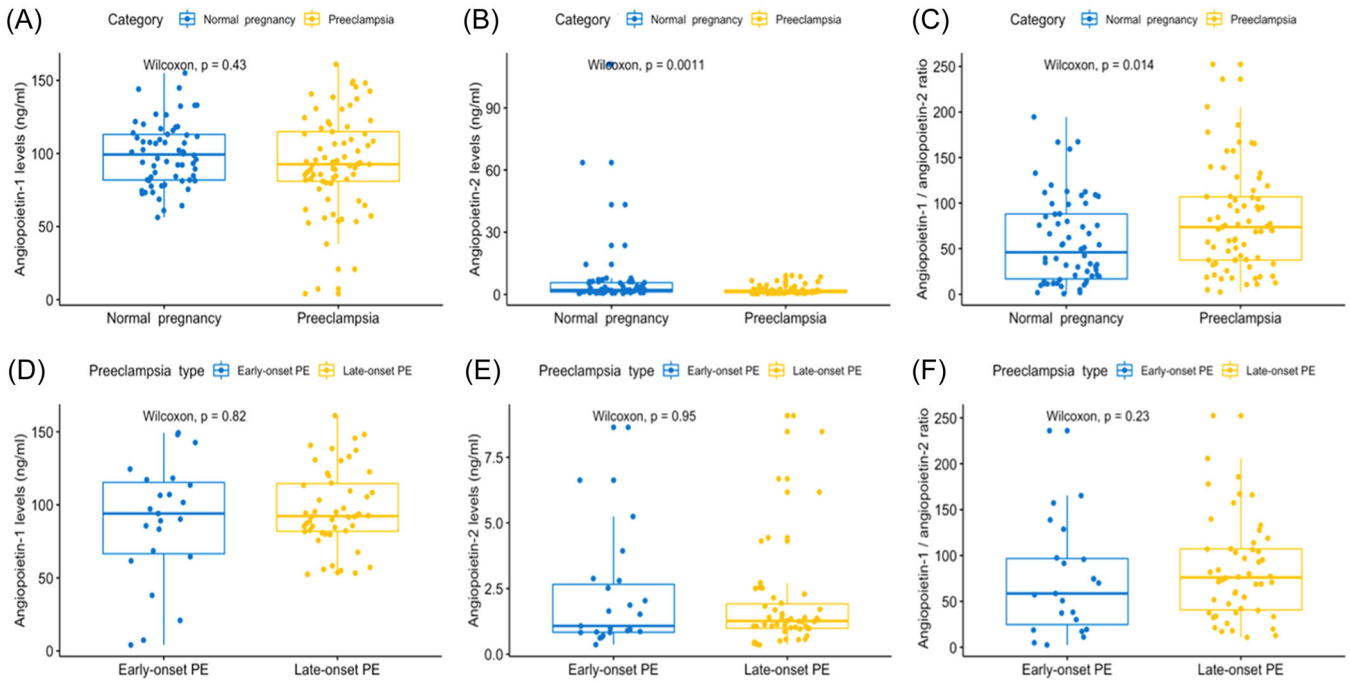

FIGURE 1 (A–F) Maternal serum angiopoietin levels in pre-eclampsia and normotensive pregnancy.

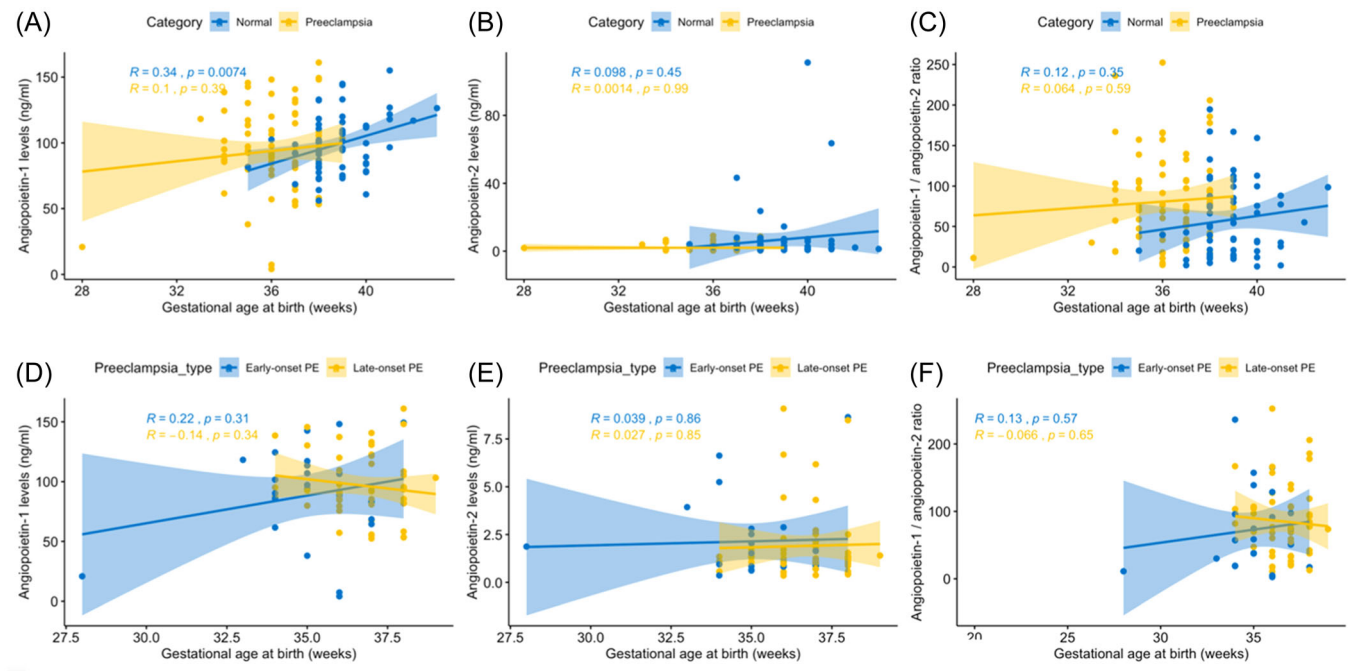

FIGURE 2 (A–F) Correlation between angiopoietin levels and gestational age at birth in pre-eclampsia and normotensive pregnancy.

differences in angiopoietin levels may indicate that the pathophysiological mechanisms underlying PE, particularly concerning angiogenesis and vascular function, are complex and multifactorial. Additional investigations are warranted to better understand the underlying mechanisms and identify more accurate predictors of disease severity and onset.

To buttress this point, unlike Ang-1, Ang-2 is a strong mitogen whose activity appears to complement the function of VEGF in normal pregnancy by rendering endothelial cells more accessible to VEGF.[13,24,26] In contrast, a case-control study conducted at the Helsinki University Hospital by Leinonen et al.,[11] reported that at 12–15 weeks of gestation, maternal serum Ang-1 and Ang-2 were high, and elevated serum levels of Ang-2 occurred at 16–20 weeks of gestation in women who subsequently developed PE.[27]

Furthermore, Ang-1/Ang-2 ratio was significantly higher in women with PE compared to normotensive pregnancy. This finding

TABLE 4 Pregnancy outcomes and maternal serum angiopoietin levels in pre-eclampsia.

Pregnancy outcome	Ang-1 Median (IQR)	*p*-Value	Ang-2 Median (IQR)	*p*-Value	Ang-1/Ang-2 ratio Median (IQR)	*p*-Value
Mode of delivery						
Vaginal	104.36 (84.97, 131.66)	0.02	1.35 (1.12, 2.28)	0.75	72.20 (46.46, 90.57)	0.78
Cesarean	90.22 (68.53, 109.55)		1.08 (0.83, 2.15)		74.57 (37.62, 118.80)	
Preterm birth						
Yes	93.32 (85.10, 115.33)	0.95	1.28 (0.87, 2.16)	0.96	72.61 (35.38, 120.71)	0.63
No	91.96 (68.53, 114.92)		1.25 (0.98, 2.15)		74.57 (42.17, 106.22)	
Low-birth weight						
Yes	91.96 (82.17, 113.48)	0.83	1.25 (0.94, 2.29)	0.89	80.05 (37.33, 113.84)	0.75
No	93.18 (74.73, 117.46)		1.26 (0.88, 2.09)		70.73 (39.96, 102.35)	
DBP classification						
Mild HPN at birth	90.72 (80.92, 113.46)	0.28	1.38 (1.01, 2.54)	0.26	64.46 (932.50, 106.99)	0.59
Severe HPN at birth	94.04 (79.91, 118.22)		1.13 (0.84, 1.87)		81.89 (40.13, 118.80)	
SBP classification						
Mild HPN at birth	91.96 (79.59, 113.48)	0.46	1.25 (0.96, 2.80)	0.49	68.93 (33.42, 127.57	0.73
Severe HPN at birth	93.09 (80.92, 117.18)		1.28 (0.87, 1.94)		75.04 (37.76, 106.22)	
DBP or SBP classification						
Mild HPN at birth	85.58 (57.20, 109.55)	0.71	1.26 (0.96, 2.88)	0.61	61.62 (21.32, 113.84)	0.89
Severe HPN at birth	94.04 (81.79, 117.18)		1.25 (0.87, 2.04)		74.57 (38.19, 107.33)	

Abbreviations: Ang-1, angiopoietin 1; Ang-2, angiopoietin 2; DBP, diastolic blood pressure; HPN, hypertension; IQR, interquartile range; SBP, systolic blood pressure.

is in contradiction with that of Bolin et al. who reported a significantly low median Ang-1/Ang-2 ratio between 25 and 28 weeks in pregnant women who later developed PE compared to normotensive pregnant women.

Schneuer et al.[12] in Australia reported that high Ang-1/Ang-2 ratios were significantly associated with adverse pregnancy outcomes although maternal and clinical risk factors showed improved predictive accuracy. In our study, women who delivered vaginally had significantly high maternal serum levels of Ang-1 when compared to women who had cesarean section but there was no association between Ang-2 or Ang-1/Ang-2 ratio and pregnancy outcomes. The finding of higher Ang-1 levels in women who delivered vaginally suggests a potential role of Ang-1 in the physiological processes related to labor and vaginal delivery.

It is noteworthy that no significant associations were observed between Ang-2 levels or the Ang-1/Ang-2 ratio and pregnancy outcomes (preterm birth, birth weight, and severity of hypertension). This suggests that while Ang-2 and the Ang-1/Ang-2 ratio are known to be important in maintaining vascular integrity, their specific levels in maternal serum may not directly correlate with adverse pregnancy outcomes assessed in this study. The lack of association between Ang-2 or the Ang-1/Ang-2 ratio and pregnancy outcomes could be attributed other confounding factors including gestational age, underlying maternal conditions, or obstetric interventions.

4.3 | Strengths and limitations

The strengths of this study include the use of a case-control design resulting in the direct comparison between angiopoietin levels in PE and pregnant normotensives. Also, the adoption of the ACOGs criteria in defining PE is considered a strength as it is widely accepted. Furthermore, the findings of the study provide significant insight into maternal serum angiopoietin levels and their potential association with pregnancy outcomes. Other factors, such as genetic variations, environmental factors, and maternal health conditions, could also influence the development of PE and pregnancy outcomes. Therefore, the generalizability of the findings is interpreted in the context of these potential confounding factors. While the study provides insights into maternal serum angiopoietin levels and PE, its generalizability is limited by the specific population studied, small sample size and different gestational ages at recruitment of the study participants. Further research involving larger and more diverse populations is needed to confirm and extend these findings.

5 | CONCLUSION

The study has highlighted the association between maternal serum angiopoietin levels in PE and pregnancy outcomes. Maternal serum level of Ang-2 was significantly lower in PE than in normotensive pregnancy. There was no significant difference in serum level of Ang-1 between PE and normal pregnancies. There was a significant positive correlation between serum Ang-1 levels and gestational age at birth in normotensive pregnancy but not in PE. Ang-1/Ang-2 ratio was significantly elevated among women with PE compared to normotensive pregnancies but did not have a significant impact on pregnancy outcome. The findings of this study highlight the contribution of angiopoietins to the pathophysiology of PE and pregnancy outcomes. We recommend further research to explore the prediction of PE and pregnancy outcomes based on the assessment of angiopoietins in early pregnancy. While this study provides insights into the association between Ang-1 levels and the mode of delivery, further research is needed to better understand the role of angiopoietins in pregnancy outcomes. Future studies could explore the dynamics of Ang-1 and Ang-2 levels throughout pregnancy, examine additional biomarkers related to vascular function, and consider a more comprehensive assessment of pregnancy outcomes.

AUTHOR CONTRIBUTIONS

Fidelis Bayor: Conceptualization; formal analysis; investigation; methodology; writing—original draft. **Kwame Adu-Bonsaffoh**: Conceptualization; data curation; formal analysis; qinvestigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review and editing. **Charles Antwi-Boasiako**: Conceptualization; investigation; methodology; project administration; supervision; validation; visualization; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

TRANSPARENCY STATEMENT

The lead author Kwame Adu-Bonsaffoh affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available but are obtainable from the corresponding author on reasonable request.

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