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Original Research

Maternal Plasma Levels of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 as Predictive Biomarkers of Preeclampsia

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Abstract

Background: Preeclampsia (PE), a severe pregnancy complication, is a major contributor to maternal and perinatal morbidity and mortality worldwide. Matrix metalloproteinases (MMPs) and their tissue inhibitors of metalloproteinases (TIMPs) are implicated in the pathogenesis of PE. This study aimed to evaluate maternal plasma levels of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 in normal and preeclamptic pregnancies across gestational trimesters to assess their predictive value. **Methods**: This case-control study included a total of 138 pregnant women: 72 with normal pregnant women (controls) and 66 women diagnosed with PE, recruited at Hanoi Obstetrics and Gynecology Hospital between July 2022 and June 2024. In the second trimester (14–28 weeks), the PE and control groups included 14 and 18 women, respectively. In the third trimester (29–41 weeks), the study included 52 women with PE and 54 controls. Plasma concentrations of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 were measured using enzyme-linked immunosorbent assay (ELISA). Correlations between MMPs, TIMPs, MMP/TIMP ratios, and PE severity indices were analyzed using Spearman's correlation. Receiver operating characteristic (ROC) analysis evaluated the diagnostic and predictive utility of these markers. **Results**: Maternal plasma levels of MMP-2 and TIMP-2 were significantly higher in the PE group compared to controls during both the second and third trimesters ($p < 0.05$). The MMP-2/TIMP-2 ratio was significantly lower in the PE group during the third trimester ($p = 0.002$). MMP-3 levels were markedly elevated in PE patients ($p < 0.001$) and were positively correlated with plasma creatinine and proteinuria. In contrast, MMP-9 levels were significantly reduced in the PE group across both trimesters ($p < 0.05$), whereas TIMP-1 levels showed no significant difference between groups. Notably, MMP-9 was strongly correlated with TIMP-1 in PE, and both markers correlated with systolic blood pressure (SBP). ROC curve analysis indicated the combination of MMP-2 and TIMP-2 as the best predictor of PE in the second trimester, while TIMP-2 was the most predictive marker in the third trimester. **Conclusions**: Elevated plasma MMP-2 and TIMP-2 levels, particularly in the second trimester, show potential as biomarkers for identifying patients at risk of PE. TIMP-2 appears promising for prediction in the third-trimester. These findings warrant further investigation for potential clinical application.

Keywords: preeclampsia; matrix metalloproteinase-2 (MMP-2); MMP-3; MMP-9; tissue inhibitor of metalloproteinase-1 (TIMP-1); TIMP-2; biomarker

1. Introduction

Preeclampsia (PE) is a complex, multi-organ disorder typically emerging after 20 weeks of gestation or postpartum, characterized by new-onset hypertension and proteinuria or significant end-organ dysfunction [1]. Although its etiology is not fully understood, a widely accepted two-stage model proposes that inadequate trophoblast invasion and disordered uterine spiral artery remodeling lead to placental ischemia/hypoxia, followed by systemic maternal endothelial dysfunction driven by an imbalance of circulating angiogenic and anti-angiogenic factors [2].

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases crucial for extracellular matrix (ECM) remodeling during reproductive processes, including gametogenesis, implantation, angiogenesis, placentation, and parturition. MMP activity is tightly regulated transcriptionally, via pro-enzyme activation, and through inhibition by tissue inhibitors of metalloproteinases (TIMPs). Dysregulation of the MMP/TIMP balance has been implicated in cardiovascular disorders, including PE [3].

This study focused on a key group of MMPs involved in trophoblastic invasion into the maternal uterine and vascular remodeling, specifically MMP-2 (Gelatinase A), MMP-9 (Gelatinase B), and MMP-3 (Stromelysin-1), along with their primary endogenous inhibitors, TIMP-2 (inhibits MMP-2) and TIMP-1 (inhibits MMP-9). MMP-2 and MMP-9 degrade various ECM components, including collagen types IV, V, VII, and X, as well as gelatin. MMP-3 targets collagen, proteoglycans, laminin, and fi-

bronectin [3]. MMP-2 plays a vital role in implantation, as well as systemic and uterine vasodilation, MMP-9 in vascular basement membrane remodeling, and MMP-3 in trophoblast motility and activation of other MMPs, like MMP-1, MMP-7, and MMP-9, thereby amplifying ECM degradation [3,4].

Altered levels of MMPs and TIMPs have been reported in the plasma, amniotic fluid, and endothelial cells of women with PE, suggesting a potential role in the disease's pathogenesis through both uteroplacental and vascular dysfunction. However, prior studies have evaluated individual MMPs or TIMPs at specific time points during pregnancy, without accounting for their dynamic changes throughout gestation or their regulatory interactions [5–7]. This limitation reduces the translational value of such findings. In the present study, we measured plasma concentrations of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2, as well as the MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios, in both normal and preeclamptic pregnant women during the second and third trimesters. We further assessed their associations with clinical indicators of PE and performed receiver operating characteristic (ROC) curve analysis to determine their trimester-specific predictive value.

2. Materials and Methods

2.1 Study Design and Subjects

A case-control study was conducted at Hanoi Obstetrics & Gynecology Hospital from July 2022 to June 2024. A total of 138 pregnant women of reproductive age (15–49 years) were included: 66 were diagnosed with PE and 72 normal pregnant women matched for age and gestational age, serving as the control group. Within the PE group, 14 women were in the second trimester (14–28 weeks) and 52 in the third trimester (29–41 weeks). The control group included 18 women in the second trimester and 54 in the third trimester. All participants provided written informed consent in accordance with institutional guidelines. The study protocol was approved by the Hanoi Obstetrics and Gynecology Hospital Ethics Committee (Approval No. 734 CN/BVPS – TT ĐT CĐT).

Inclusion Criteria (PE Group): Gestational age >20 weeks with a PE diagnosis according to the American College of Obstetricians and Gynecologists (ACOG) 2020 criteria [1].

Exclusion Criteria (All Participants): Pregnancies resulting from assisted reproductive technologies, multiple gestations, fetal structural or chromosomal abnormalities, stillbirths, and pre-existing maternal conditions such as chronic hypertension, cardiovascular disease, liver disease, renal disorders, endocrine disorders (e.g., diabetes, Graves' disease), neoplastic conditions, autoimmune diseases, and significant infectious diseases (e.g., human immunodeficiency virus [HIV], hepatitis) or other acute infections.

2.2 Clinical Data Collection

Demographic data (maternal age, gestational age, obstetric history including gravidity and prior PE), ongoing medical treatments, blood pressure, and symptoms (headache, blurred vision, edema) were recorded. Laboratory assessments included complete blood count (red blood cell [RBC], hemoglobin, platelets), plasma biochemistry (aspartate aminotransferase [AST], alanine aminotransferase [ALT], urea, creatinine, uric acid, albumin), and 24-hour proteinuria.

2.3 Sample Collection and Processing

A single 2–5 mL peripheral blood sample was collected from each participant into ethylenediamine tetraacetic acid (EDTA) (0222, MPV Medical Plastic Joint Stock Company, Tam Diep, Ninh Binh, Vietnam) anticoagulant tubes at the time of hospital admission, before the initiation of any antihypertensive or other treatments. Plasma was separated by centrifugation (5000 rpm, 5 min) and stored at –80 °C until analysis.

2.4 Enzyme-linked Immunosorbent Assay (ELISA) Measurements

Plasma concentrations were measured using sandwich ELISA kits from Proteintech Group (Rosemont, IL, USA) as follows: Human MMP-2 (Catalogue No.KE00077), Human MMP-3 (Catalogue No.KE00160), Human MMP-9 (Catalogue No.KE00164), Human TIMP-1 (Catalogue No.KE00166), and Human TIMP-2 (Catalogue No.KE00162). Absorbance was measured at 450 nm (correction wavelength 630 nm) using a BioTek ELx808 Microplate Reader (Agilent BioTek, Winooski, VT, USA) operated with BioTek Gen5 software (version 3.05, Agilent BioTek, Winooski, VT, USA).

The analytical performance parameters for each assay were as follows: For MMP-2, the range was 0.625–20 ng/mL, with an intra-assay coefficient of variation (CV) 4.5%–10% and an inter-assay CV 5.4%–8.9%. For MMP-3, the range was 31.25–2000 pg/mL, with intra-assay CV 4.6%–8.6% and inter-assay CV 6.2%–9.2%. For MMP-9, the range was 62.5–4000 pg/mL, with intra-assay CV 1.5%–3.0% and inter-assay CV 3.2%–6.7%. For TIMP-1, the range was 62.5–4000 pg/mL, with intra-assay CV 4.1%–6.2% and inter-assay CV 7.5%–8.4%. For TIMP-2, the range was 31.25–2000 pg/mL, with intra-assay CV 3.1%–4.1% and inter-assay CV 2.7%–3.2%.

2.5 Statistical Analysis

Data analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Kolmogorov-Smirnov test. Non-normally distributed data were presented as median and interquartile range (IQR: 1st quartile; 3rd quartile), compared between groups (PE vs. Control), and across trimesters within groups using the Mann-Whitney U-test. Spearman's rank

Table 1. General characteristics of the study subjects.

Parameters	Total (n = 138)				Second trimester (n = 32)				Third trimester (n = 106)			
	Control group (n = 72)	PE group (n = 66)	Z	*p*	Control group (n = 18)	PE group (n = 14)	Z	*p*	Control group (n = 54)	PE group (n = 52)	Z	*p*
Maternal age (years)	29 (26–32)	30 (26–33)	–0.6	0.567	29 (27–33)	33 (29–36)	–1.4	0.153	29 (26–32)	29 (25–32)	–0.1	0.977
Gestational age (weeks)	36 (28–39)	35 (29–38)	–0.6	0.601	26 (23–27)	26 (24–27)	–0.5	0.604	38 (33–39)	37 (33–39)	–1.4	0.150
Gravidity	2 (1–3)	2 (1–3)	–0.1	0.944	2 (1–2)	2 (1–3)	–0.5	0.667	2 (1–3)	2 (1–2)	–0.3	0.733
SPB (mmHg)	110 (110–110)	150 (140–160)	–10.6	<0.001	110 (110–110)	155 (140–163)	–5.0	<0.001	110 (110–110)	150 (140–155)	–9.3	<0.001
DBP (mmHg)	70 (70–70)	100 (90–100)	–10.6	<0.001	70 (70–70)	100 (90–100)	–5.0	<0.001	70 (70–70)	90 (90–100)	–9.2	<0.001
MAP (mmHg)	83 (83–83)	113 (107–120)	–10.4	<0.001	83 (83–83)	119 (109–130)	–5.0	<0.001	83 (83–83)	112 (107–119)	–9.1	<0.001
Creatinine (μmol/L)	57 (51–63)	66 (59–77)	–5.6	<0.001	51 (50–54)	65 (57–82)	–4.0	<0.001	59 (53–66)	67 (59–76)	–4.2	<0.001
Platelets (G/L)	236 (193–259)	217 (180–264)	–0.8	0.411	260 (231–292)	249 (182–280)	–1.1	0.287	222 (188–251)	215 (177–260)	–0.3	0.781
AST (U/L)	18 (15–20)	23 (19–36)	–5.6	<0.001	18 (15–19)	25 (22–42)	–4.0	<0.001	18 (15–20)	22 (18–36)	–4.3	<0.001
ALT (U/L)	12 (9–16)	15 (12–32)	–4.0	<0.001	11 (10–16)	19 (15–36)	–3.3	0.001	12 (8–16)	14 (11–30)	–2.9	0.004
Proteinuria (g/L)	0 (0–0)	2 (1–8)	–10.2	<0.001	0 (0–0)	4 (1–9)	–4.6	<0.001	0 (0–0)	2 (1–6)	–9.0	<0.001

Note: Data presented as median (interquartile range: IQR). Mann-Whitney U-test comparing PE vs. Control within each column. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PE, Preeclampsia.

correlation coefficient (rho) assessed correlations between biomarkers and clinical/laboratory indices (systolic blood pressure [SBP], diastolic blood pressure [DBP], platelets, creatinine, AST, ALT, and proteinuria). ROC curves were generated for significant markers to evaluate their predictive value for PE, calculating the area under the curve (AUC).

3. Results

3.1 Comparison of General Clinical Data

Among 138 participants (32 in the second trimester, 106 in the third trimester), there were no statistically significant differences in maternal age or gestational age between the PE and control groups ($p > 0.05$). SBP, DBP, and mean arterial pressure (MAP) were significantly higher in the PE group ($p < 0.001$). Plasma AST, ALT, creatinine, and proteinuria levels were also significantly elevated in the PE group compared to controls ($p < 0.001$ or $p < 0.01$), with proteinuria particularly pronounced in the second trimester. Platelet counts were insignificantly lower in the PE group overall ($p = 0.411$), and remained within the normal range for most individuals (Table 1).

3.2 Comparison of MMP and TIMP Plasma Levels

Plasma MMP-2 and MMP-3 levels were significantly higher in the PE group than in controls in both trimesters and overall ($p < 0.05$ for all). Plasma MMP-9 levels were significantly lower in the PE group compared to the control group in both trimesters and overall ($p < 0.05$ for all). Plasma TIMP-1 levels did not differ significantly between groups in third trimester or overall. Plasma TIMP-2 levels were significantly higher in the PE group in both trimesters and overall ($p < 0.01$ for all). The MMP-9/TIMP-1 ratio was significantly lower in the PE group in overall ($p < 0.001$) and in both trimesters ($p < 0.05$). The MMP-2/TIMP-2 ratio was significantly lower in the PE group in the third trimester and overall ($p = 0.002$), but not in the second trimester ($p = 0.621$). The MMP-2/MMP-9 ratio was significantly higher in the PE group in both trimesters and overall ($p < 0.05$) (Table 2). All MMPs, TIMPs levels, and their ratios in each group did not differ between the second and third trimesters ($p > 0.05$) (**Supplementary Table 1**).

3.3 Correlations Between Biomarkers and Clinical Indices in the PE Group

A strong positive correlation was observed between MMP-9 and TIMP-1 levels ($r = 0.773$, $p < 0.001$). A weak positive correlation was found between MMP-9 and SBP ($r = 0.288$, $p = 0.019$). A weak negative correlation was observed between TIMP-2 and ALT ($r = –0.249$, $p = 0.044$). A moderate positive correlation was observed between TIMP-1 and SBP ($r = 0.403$, $p = 0.001$). MMP-3 showed positive correlation with creatinine levels ($r = 0.286$, $p = 0.020$) and with proteinuria levels ($r = 0.365$, $p = 0.003$) (Table 3).

3.4 ROC Curve Analysis for PE Prediction in the Second Trimester

In the second trimester, ROC analysis showed that MMP-2 (AUC = 0.817), TIMP-2 (AUC = 0.810), the MMP-2/MMP-9 ratio (AUC = 0.853), and the combination of MMP-2 and TIMP-2 (AUC = 0.893) were effective predictors of PE (all AUC >0.8, $p < 0.01$). The combination model (MMP-2 ≥260.8 ng/mL and TIMP-2 ≥415.6 ng/mL) yielded the highest AUC, with 85.7% sensitivity and 88.9% specificity. MMP-3 showed fair predictive value (AUC = 0.798) (Fig. 1, Table 4).

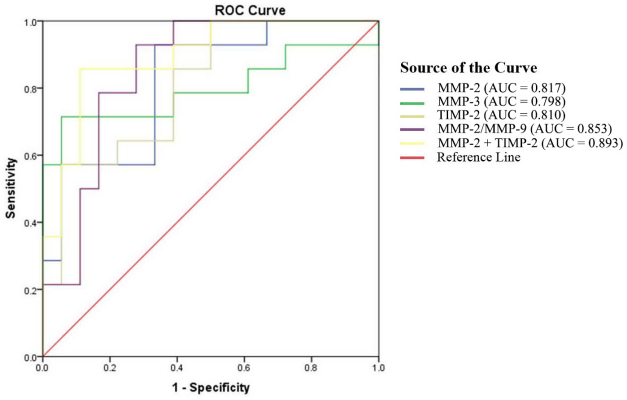

Fig. 1. The ROC curves for plasma concentrations of MMPs and TIMPs in PE during the second trimester. ROC, receiver operating characteristic; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; AUC, area under the curve.

3.5 ROC Curve Analysis for PE Prediction in the Third Trimester

In the third trimester, TIMP-2 demonstrated the best predictive performance (AUC = 0.807, $p < 0.001$). The optimal cut-off for TIMP-2 was ≥305.8 ng/mL, yielding 67.3% sensitivity and 79.6% specificity. MMP-3 (AUC = 0.714), the combination of MMP-2 and TIMP-2 (AUC = 0.797) also showed fair predictive ability (AUC >0.7, $p < 0.001$). Other markers, including MMP-2, MMP-2/MMP-9 ratio, had lower predictive values (AUC <0.7) (Fig. 2, Table 5).

4. Discussion

MMP-2 (Gelatinase A), expressed by decidual cells and trophoblasts, degrades key ECM components. It is secreted in a latent form (pro-MMP-2), and its activation involves TIMP-2 and MT1-MMP (MMP-14) in a complex process in which TIMP-2 can both facilitate activation and inhibit active MMP-2. MMP-2 is crucial for early implantation, and systemic and uterine vasodilation to ensure adequate fetal perfusion, but its activity is tightly regulated by TIMP-2, maintaining a balance essential for normal placentation [3,4,8].

Table 2. Comparison of MMP and TIMP plasma levels between groups.

Indicators	Gestation	Control group (n = 72)		PE group (n = 66)		Z	*p*
		Median	IQR	Median	IQR		
MMP-2 (ng/mL)	Second trimester	250.2	206.6–369.4	444.3	308.0–604.6	–3.0	0.002
	Third trimester	270.6	136.8–407.7	360.5	255.1–566.0	–2.3	0.022
	Total	254.2	168.9–379.3	368.5	249.7–562.5	–3.3	0.001
MMP-3 (ng/mL)	Second trimester	17.9	12.4–23.7	38.5	19.2–91.2	–2.9	0.004
	Third trimester	17.5	11.2–24.0	26.2	17.9–48.2	–3.8	<0.001
	Total	17.7	11.7–23.8	27.3	18.1–57.5	–4.7	<0.001
MMP-9 (ng/mL)	Second trimester	839.0	381.6–1254.2	241.5	107.6–737.8	–2.3	0.023
	Third trimester	608.6	241.2–1269.6	372.8	215.1–625.9	–2.0	0.044
	Total	653.8	266.6–1206.4	352.2	194.1–637.5	–3.0	0.003
TIMP-1 (ng/mL)	Second trimester	240.1	200.1–259.7	132.4	85.9–217.2	–2.0	0.048
	Third trimester	242.3	137.2–340.0	193.3	127.3–276.5	–1.2	0.237
	Total	242.3	145.0–319.0	188.7	120.6–273.4	–1.9	0.059
TIMP-2 (ng/mL)	Second trimester	235.4	170.2–309.2	456.9	288.1–539.9	–3.0	0.003
	Third trimester	213.3	71.3–296.3	364.8	273.9–500.0	–5.5	<0.001
	Total	218.4	144.3–299.6	388.0	276.7–511.4	–6.3	<0.001
MMP-2/TIMP-2	Second trimester	1.5	0.7–1.7	1.1	0.8–1.5	–0.5	0.621
	Third trimester	1.6	0.8–3.9	1.1	0.6–1.7	–3.2	0.002
	Total	1.6	0.8–2.9	1.1	0.7–1.6	–3.0	0.002
MMP-9/TIMP-1	Second trimester	4.2	2.5–4.9	1.7	1.3–3.1	–2.8	0.006
	Third trimester	3.0	1.6–4.3	2.1	1.3–2.9	–2.4	0.015
	Total	3.2	1.9–4.5	2.0	1.3–2.9	–3.5	<0.001
MMP-2/MMP-9	Second trimester	0.4	0.2–0.8	1.4	0.9–3.1	–3.4	0.001
	Third trimester	0.5	0.2–0.9	0.9	0.5–2.3	–3.2	0.002
	Total	0.5	0.2–0.9	1.1	0.6–2.3	–4.4	<0.001

Note: MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase. Mann-Whitney U-test comparing PE vs. Control group.

Table 3. Spearman's correlations between biomarkers and clinical indices in the PE group (n = 66).

	MMP-2		MMP-3		MMP-9		TIMP-1		TIMP-2	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
SBP	0.124	0.321	0.092	0.463	0.288	0.019	0.403	0.001	0.051	0.686
DBP	–0.007	0.958	–0.074	0.553	0.124	0.322	0.238	0.054	–0.047	0.706
Platelets	–0.142	0.255	–0.169	0.174	–0.026	0.835	0.046	0.712	–0.026	0.838
Creatinine	0.204	0.101	0.286	0.020	0.134	0.284	0.058	0.646	–0.079	0.530
AST	0.208	0.093	0.103	0.413	0.012	0.925	0.069	0.580	–0.115	0.357
ALT	0.140	0.262	0.013	0.918	0.171	0.169	0.188	0.131	–0.249	0.044
Proteinuria	0.239	0.054	0.365	0.003	0.166	0.182	0.157	0.199	0.108	0.388
MMP-2			0.159	0.203	0.038	0.76	0.083	0.505	0.124	0.320
MMP-3					0.175	0.160	0.109	0.382	0.196	0.115
MMP-9							0.773	<0.001	0.087	0.489
TIMP-1									0.023	0.857

Note: Statistically significant correlations ($p < 0.05$).

Our finding of significantly increased plasma MMP-2 and TIMP-2 levels in PE patients compared to controls in both trimesters and overall, aligns with previous studies [9,10]. However, the MMP-2/TIMP-2 ratio declined significantly in the PE group in the third trimester and overall, which may reflect its role in PE pathogenesis. Placental ischemia/hypoxia may stimulate vascular endothelial growth factor (VEGF) production, subsequently upregulating MMP-2 expression [11]. Elevated MMP-2 may also be mediated by interleukin-8 (IL-8) from endothelial cells under a chronic inflammatory conditions [12]. However, the reduced MMP-2/TIMP-2 ratio might indicate decreased proteolytic potential of MMP-2, resulting in impaired trophoblast invasion and vascular remodeling [3]. Addition-

Table 4. ROC analysis results for predicting PE during the second trimester.

Indicators	AUC	95% CI	Cut-off	Youden index	Sensitivity	Specificity	*p*
MMP-2 (ng/mL)	0.817	0.670–0.965	260.8	0.595	92.9	66.7	0.002
MMP-3 (ng/mL)	0.798	0.619–0.976	27.7	0.659	71.4	94.4	0.004
TIMP-2 (ng/mL)	0.810	0.661–0.958	415.6	0.516	57.1	94.4	0.003
MMP-2/MMP-9	0.853	0.718–0.988	0.6	0.651	92.9	72.2	0.001
Combination of MMP-2 and TIMP-2 (ng/mL)	0.893	0.781–1.000	MMP-2: 260.8 TIMP-2: 415.6	0.746	85.7	88.9	<0.001

Note: AUC, area under the curve; CI, confidence interval.

Table 5. ROC analysis results for predicting PE during the third trimester.

Indicators	AUC	95% CI	Cut-off	Youden index	Sensitivity	Specificity	*p*
MMP-2 (ng/mL)	0.629	0.524–0.735	397.5	0.202	46.2	74.1	0.022
MMP-3 (ng/mL)	0.714	0.617–0.811	20.4	0.322	69.2	63.0	<0.001
TIMP-2 (ng/mL)	0.807	0.726–0.888	305.8	0.469	67.3	79.6	<0.001
MMP-2/MMP-9	0.678	0.575–0.728	0.7	0.340	69.2	64.8	0.002
Combination of MMP-2 and TIMP-2 (ng/mL)	0.797	0.714–0.880	MMP-2: 397.5 TIMP-2: 305.8	0.472	75.0	72.2	<0.001

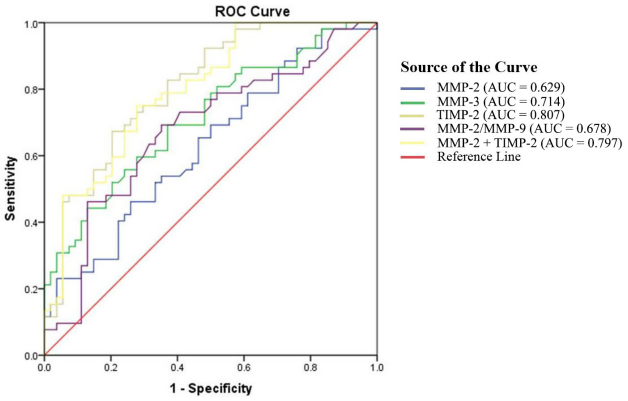

Fig. 2. The ROC curves for plasma concentrations of MMPs and TIMPs in PE during the third trimester.

ally, the MMP-2 dependent vasodilatory effect is even more pronounced in PE compared to normal pregnancies or non-pregnant women. This suggests that inhibition of MMP-2 by TIMP-2 may decrease its vasodilatory capacity and increase the risk of vasoconstriction, exacerbating hypertensive symptoms [8]. Thus, altered MMP-2/TIMP-2 balance appears linked to both early and later stages of PE.

Notably, MMP-2 showed strong predictive value for PE in the second trimester (AUC = 0.817), consistent with findings by Bahabayi *et al*. [13], although our optimal cut-off (260.8 ng/mL) differed slightly, potentially due to population variations. Combining MMP-2 with TIMP-2 further improved second-trimester prediction (AUC = 0.893). In the third trimester, TIMP-2 emerged as the best single predictor (AUC = 0.807), supported by studies linking TIMP-2 (often combined with insulin-like growth factor binding protein-7 [IGFBP7]) to PE-related acute kidney injury [14].

MMP-3 (Stromelysin-1) is secreted in a zymogen form (pro-MMP-3) by decidual stromal cells, macrophages, and extravillous trophoblasts. Pro-MMP-3 is activated by serine proteases like trypsin and plasmin via proteolytic cleavage of its propeptide domain. During pregnancy, MMP-3 facilitates the directional migration of trophoblasts into maternal tissues by cleaving IGFBP-1, a key regulator of trophoblast motility. Additionally, MMP-3 contributes to ECM remodeling both directly, by degrading ECM components, and indirectly, by activating other MMPs [4,15].

We found significantly elevated MMP-3 levels in patients with PE throughout pregnancy, consistent with previous studies [6,16]. A proposed pathogenesis involves stressed syncytiotrophoblasts secreting pro-inflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α), which upregulate *MMP-3* gene expression by inhibiting histone deacetylation, and simultaneously enhance the enzymatic activity of serine proteases, thereby promoting the conversion of pro-MMP-3 to its active form. Elevated MMP-3 might contribute to or reflect both maternal and fetal complications in early-onset PE, such as fetal growth restriction, lower gestational age at birth, and lower birth weight [6,16]. In this study, we also observed that MMP-3 correlated positively with plasma creatinine and proteinuria level, suggesting a link to renal involvement in PE. However, these mechanistic insights require further investigation and validation using functional assays *in vitro* or *in vivo*.

MMP-3 contributed significantly to PE prediction in the second trimester with high specificity. Although trimester-specific MMP-3 data are limited, Laskowska [6] also suggested its relevance for early-onset PE, a form of the disorder associated with placental dysfunction.

MMP-9, expressed at the maternal-fetal interface, degrades ECM components and activates VEGF, playing

roles in angiogenesis, particularly in microvascular development and tissue remodeling during labor [7,17]. Its activity is inhibited by TIMP-1, and the MMP-9/TIMP-1 balance regulates trophoblast invasion [8].

We observed significantly lower plasma MMP-9 levels and a lower MMP-9/TIMP-1 ratio in the PE group, while TIMP-1 levels were not significantly different. This finding contrasts with studies reporting increased TIMP-1, but aligns with others demonstrating decreased placental MMP-9 expression and lower MMP-9/TIMP-1 ratios in hypertensive pregnancies [7,18]. The strong positive correlation between MMP-9 and TIMP-1 ($r = 0.773$) in our PE group suggests a potential coordinated regulation or compensatory mechanism. Reduced MMP-9 activity could impair vascular remodeling and excess collagen deposition, leading to abnormal spiral artery development and contributing to placental ischemia. Syncytiotrophoblast stress resulting from ischemia might further suppress MMP-9 expression via inflammatory mediators, potentially creating a detrimental feedback loop [3]. Consistent with studies in hypertension, we found positive correlations between MMP-9, TIMP-1, and SBP in PE patients [18,19].

We evaluated the trimester-specific predictive value of MMP-9, TIMP-1, and the MMP-9/TIMP-1 ratio; however, their performances were limited (AUC <0.5). Interestingly, the MMP-2/MMP-9 ratio demonstrated stronger predictive potential for PE during the second trimester, which is consistent with previous findings [20]. This ratio may serve as a more promising biomarker for future validation.

This study has some limitations that should be considered. First, it was conducted at a single center, which may limit the generalizability of the findings. Second, the use of a single blood sample provides only a snapshot of biomarker levels and does not reflect the dynamic changes that occur throughout pregnancy; therefore, longitudinal follow-up is necessary. Third, the study did not include functional assays such as zymography to evaluate the enzymatic activity of MMPs, which would have provided deeper mechanistic insight into their biological roles. Despite these limitations, our findings highlight trimester-specific alterations in MMPs and TIMPs in PE. The strong predictive performance of the combined MMP-2 and TIMP-2 in the second trimester, and of TIMP-2 alone in the third trimester, supports their potential utility as components of a multi-marker panel for PE risk assessment or diagnosis. While plasma analysis may not fully reflect placental events, its feasibility supports potential integration into prenatal screening protocols. Further longitudinal studies in larger and more diverse populations are needed to validate these findings and explore their clinical applicability.

5. Conclusions

Plasma levels of MMP-2, and TIMP-2, along with the MMP/TIMP ratio, are significantly altered in women with PE compared to controls, showing distinct trimester-specific patterns. Elevated MMP-2 and TIMP-2 levels, particularly their combination in the second trimester, and elevated TIMP-2 levels in the third trimester, show promise as potential predictive biomarkers for PE. These findings warrant further validation before clinical application.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

TTN and NAL designed the research study. NAL, TDD, TNDN, TNTN, and SHD performed the research. TNTN, TDD and NAL analyzed the data. TNTN and TTN drafted the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was carried out in accordance with the guidelines of the Declaration of Helsinki. This study was reviewed and approved by the Ethics Committee of the Hanoi Obstetrics & Gynecology Hospital (Approval No. 734 CN/BVPS – TT ĐT CĐT, Date: June 24, 2022). Informed consent was obtained from all individual participants included in the study.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT-4o in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG42755.

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