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Circulating levels of tissue inhibitor of metalloproteinase (TIMP)-3, a protein with inhibitory effects on angiogenesis, are increased in pre-eclampsia

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Abstract

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AUTHOR CONTRIBUTIONS

ACP, JdeOC, JLC, JCP, MR-V, VRR-V, RCC, PRN, MRL and VCS contributed to the conception, design, participant recruitment and data collection of the present study. JdeOC, PRN, MRL and VCS contributed in data analysis. ACP, JdeOC, PRN, MRL and VCS drafted the article. All authors were involved in interpreting the data and critically reviewing the article. All authors gave approval of the final version for publication.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section in the online version of this article:

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

Objective: To assess and compare circulating TIMP-3 concentrations between pre-eclampsia and healthy pregnant women. Moreover, we aimed to determine the relationships between circulating TIMP-3 with MMP-2, MMP-9, TIMP-1 and TIMP-2 concentrations in pre-eclampsia.

Methods: A primary case-control study included patients with pre-eclampsia (n=219) and gestational hypertension (n=118), healthy pregnant (n=214) and non-pregnant women (n=66), and a replication case-control study included patients with pre-eclampsia (n=177) and healthy pregnant women (n=124), both from Southeastern Brazil. Plasma TIMP-3, MMP-2, MMP-9, TIMP-1, and TIMP-2 concentrations were assessed using commercially available ELISA kits, and the relationships between them were analyzed using Spearman's correlation.

Results: In our primary study, patients with pre-eclampsia and gestational hypertension exhibited increased TIMP-3 concentrations compared to healthy pregnant (both $p<0.0001$) and non-pregnant women (both $p<0.001$). These findings were confirmed in the replication study, showing elevated TIMP-3 concentrations in pre-eclampsia versus healthy pregnant women ($p<0.001$). We found no difference in TIMP-3 concentrations between early-onset and late-onset pre-eclampsia. Moreover, TIMP-3 concentrations were significantly correlated with plasma concentrations of TIMP-1 ($r=0.2333$; $p=0.0086$) and MMP-2 ($r=0.2159$; $p=0.0156$) in pre-eclampsia.

Conclusions: Circulating TIMP-3 concentration is increased in pre-eclampsia compared to healthy pregnant women, and it is positively correlated to plasma MMP-2 and TIMP-1 concentrations in pre-eclampsia.

Keywords

Gestational hypertension; Matrix metalloproteinase-2; MMP-2; Pre-eclampsia; Tissue Inhibitor of Metalloproteinase-1; TIMP-1; Tissue Inhibitor of Metalloproteinase-3; TIMP-3

1. INTRODUCTION

Hypertensive disorders, including gestational hypertension (GH) and pre-eclampsia (PE), affect 5–10% of pregnancies worldwide [1, 2]. PE is a multisystem health condition characterized by new-onset hypertension and proteinuria or damage in other organs after 20 weeks of gestation [3]. Considering the gestational age at onset, PE can be classified as early-onset (<34 weeks of gestation) or late-onset (≥34 weeks of gestation) [4, 5].

Tissue inhibitors of metalloproteinase (TIMPs) are a family of endogenous inhibitors of matrix metalloproteinases (MMPs) composed by four members, TIMP1 to TIMP4. The *TIMP-3* gene is relatively highly expressed in different tissues [6], and it is regulated at transcriptional and post-transcriptional levels, as reviewed elsewhere [7]. TIMP-3 is unique among the four TIMPs because of its high affinity for the proteoglycans in the extracellular matrix, including all MMPs, a disintegrin and metalloproteinases (ADAMs), and ADAMTSs (ADAM with thrombospondin motifs) [7, 8]. Among its critical roles, TIMP-3 acts as regulator of uterine extracellular matrix degradation during embryo implantation [9]. Importantly, the balance between TIMPs and MMPs is the key to the stability and normal function of the extracellular matrix, which is a complex process involving the ability of the MMPs to degrade proteins of the extracellular matrix, while the TIMPs inhibit this process

[7, 10]. An imbalanced placental expression of MMPs and TIMPs and their plasma levels have been reported in hypertensive disorders of pregnancy [11, 12], including PE [13–18].

The roles of TIMP-3 in cardiovascular pathologies, namely myocardial disease, cardiac inflammation, aortic aneurysm, and atherosclerosis were reviewed elsewhere [7], and its therapeutic potential and application as a biomarker for predicting cancer progression [19]. Notably, our comprehensive analyses of DNA methylation of the *TIMP-3* promoter in placentas from early-onset PE and late-onset PE [20] confirmed that its promoter is hypomethylated and *TIMP-3* is overexpressed in placentas from PE [21, 22]. Increased *TIMP-3* mRNA levels resulting into increased TIMP-3 protein production could inhibit the activity of MMPs, thereby leading to impaired trophoblast invasion and abnormal placentation in PE. However, no previous study has examined TIMP-3 protein levels in PE or GH.

In this study, we assessed TIMP-3 concentration in plasma from women with PE in two populations, including the subgroups classified into early-onset PE and late-onset PE, and plasma from GH, healthy pregnant (HP) and non-pregnant (NP) women. Moreover, we examined the relationships between circulating TIMP-3 concentrations and MMP-2, MMP-9, TIMP-1 and TIMP-2 concentrations in plasma from women with PE, GH, and HP.

2. METHODS

2.1. Subjects

The primary case-control study was approved by the Institutional Review Board (#4682/2006, June 2006) of the University Hospital of Ribeirao Preto Medical School, University of Sao Paulo, Sao Paulo State, Brazil, and included 219 PE, 118 GH, 214 HP, and 66 NP women. The replication case-control study was approved by the Ethics Committee (#4.418.043, November 2020) of the Botucatu Medical School, Sao Paulo State, Brazil, and included 177 PE and 124 HP women. All subjects from both the case-control studies were consecutively enrolled in the outpatient clinics of their respective Departments of Obstetrics and Gynecology. All participants provided written informed consent.

GH was defined as a systolic blood pressure of 140 mm Hg or more or a diastolic blood pressure of 90 mm Hg or more, or both, on two or more measurements at least 6 hours apart after 20 weeks of gestation, in a woman with previously normal blood pressure [3, 23]. PE was defined as GH along with proteinuria or other sign of end organ damage in a woman after 20 weeks of gestation, according to the American College of Obstetricians and Gynecologists [23]. Women with pre-existing hypertension, with or without superimposed PE, were not included in the study. The exclusion criteria for all study groups were as follows: hemostatic abnormalities, chronic hypertension, cancer, multiple pregnancy, diabetes, cardiovascular, autoimmune, renal, and hepatic diseases. We conducted our studies in compliance with the principles of the Declaration of Helsinki.

2.2. Collection and preparation of plasma samples

Maternal venous blood samples were collected into tubes containing EDTA at the time of clinic attendance. No sample was collected at delivery. Plasma was obtained from

centrifugation of whole blood, and stored at −80°C until used to assess MMPs and TIMPs concentrations by the methods described below.

2.3. Plasma TIMP-3 measurement

The concentration of TIMP-3 in plasma from GH, PE, HP, and NP women was determined using the commercially available Duoset enzyme-linked immunosorbent assay (ELISA) kit (Catalog number: DY973, R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. Assay range was 62.5 – 4,000 pg/mL.

2.4. Plasma MMP-2, MMP-9, TIMP-1 and TIMP-2 measurements

Plasma MMP-2, MMP-9, TIMP-1 and TIMP-2 concentrations were measured using commercially available ELISA kits, as previously described [14–16].

2.5. Statistical analyses

Student's *t*-test, Mann-Whitney U test, and chi-square test were used as appropriate to examine the differences in clinical parameters and circulating TIMP-3 concentrations between PE, GH, HP, and NP women, having HP as the control group. Differences in circulating TIMP-3 concentrations were similarly analyzed when the PE group was classified into the subgroups of early-onset PE and late-onset PE, term and preterm PE, or PE with and without fetal intrauterine growth restriction.

The relationships between circulating TIMP-3 concentrations with plasma concentrations of MMP-2, MMP-9, TIMP-1, and TIMP-2 were analyzed using Spearman's correlation (r and p-values). GraphPad Prism 5.0 was used for statistical analysis and preparation of graphs. A value of $p < 0.05$ was considered the level of statistical significance.

3. RESULTS

The demographic and clinical characteristics of women enrolled in the primary case-control study are shown in Table 1. PE and GH showed an age higher than HP (p=0.0017 and p=0.0004, respectively), which was lower than NP women (p=0.0124 and p=0.0272, respectively). While body mass index (BMI) was not different between PE and HP women (p>0.05), it was higher in PE than in NP women (p<0.0001), and when GH was compared to HP and NP women (p=0.0136 and p<0.0001, respectively). As expected, PE exhibited higher systolic blood pressure and diastolic blood pressure than HP and NP women (p<0.0001 and p=0.0013, respectively), despite the fact that most women with PE were receiving antihypertensive therapy. GH exhibited higher systolic blood pressure than HP and NP women (all p<0.0001), but a higher diastolic blood pressure only when compared to NP women (p=0.0002). We found lower gestational age at delivery and gestational age at sampling, as well as lower newborn weight in PE than in HP (all p<0.0001). Gestational age at delivery and creatinine (P= 0.0022 and P= 0.0113, respectively) were lower in GH than in HP. Heart rate, fasting glucose, hemoglobin, and hematocrit showed no significant differences between groups (P> 0.05).

PE showed increased circulating TIMP-3 concentration compared to HP ($p<0.0001$) and NP women ($p=0.0002$) (Figure 1A). GH also showed increased circulating TIMP-3 concentration compared to HP ($p=0.0001$) and NP women ($p=0.0031$) (Figure 1A). When the PE group was classified according to different clinical presentations, we found no differences in circulating TIMP-3 levels between early-onset PE and late-onset PE ($p>0.05$; Figure 1C), between term and preterm PE, and between PE with and without intrauterine fetal growth restriction (all $p>0.05$; Figures S1A-B, respectively).

Next, we examined the relationships between circulating TIMP-3 concentrations with plasma concentrations of MMP-2, MMP-9, TIMP-1 and TIMP-2 in the primary case-control study. Notably, we found significant positive correlations between TIMP-3 and TIMP-1 ($r=0.2333$; $p=0.0086$) and between TIMP-3 and MMP-2 ($r=0.2159$; $p=0.0156$) concentrations in PE (Figure 2A and 2B, respectively). Moreover, we found significant positive correlations between TIMP-3 and TIMP-1 concentrations in GH ($r=0.2023$; $p=0.0254$; Figure 2C), and between TIMP-3 and TIMP-2 concentrations in HP ($r=0.1817$; $p=0.0098$) (Figure 2D). Conversely, we found no significant correlations between TIMP-3 with plasma concentrations of MMP-9 and TIMP-2 in PE, GH, and HP groups (all $p>0.05$).

To assess the relationship between circulating TIMP-3 concentrations with clinical characteristics, we performed correlation analyses with blood pressure levels, BMI, and gestational age at sampling and gestational age at delivery. However, we found no significant correlations between TIMP-3 concentrations and systolic blood pressure, diastolic blood pressure, and BMI in any of the study groups (all $p>0.05$; Figure S2A-F, and Figure S3A-C). Finally, we also found no significant correlations between TIMP-3 and gestational age at sampling or gestational age at delivery (data not shown).

The demographic and clinical characteristics of women enrolled in the replication case-control study are shown in Table 2, and they show similar trends as those described for the primary case-control study. Notably, in the replication study, PE also showed increased circulating TIMP-3 concentration compared to HP ($p=0.0004$) (Figure 1B). Again, we found no differences in the circulating TIMP-3 concentrations between early-onset PE and late-onset PE in the subjects of the replication study ($p>0.05$) (Figure 1D).

4. DISCUSSION

The main novel findings reported here are that circulating TIMP-3 concentrations are increased in PE and GH compared with HP in a case-control study, and this observation of increased TIMP-3 concentrations in PE is replicated in a different population of pregnant women. Moreover, we found that circulating TIMP-3 concentrations are positively correlated with plasma MMP-2 and TIMP-1 concentrations in PE.

A balance between TIMPs and MMPs is needed for normal function of the extracellular matrix, an essential process for the remodeling of spiral arteries and establishment of proper placentation. Therefore, we have examined the relationship of TIMP-3 with MMPs and other TIMPs. Although we have found a significant association between TIMP-3 and MMP-2 and TIMP-1 in PE, we have not conducted functional studies proving the

interaction between these proteins in placental tissue and its impact on trophoblast invasion and vascular remodeling. Nonetheless, these interesting findings serve as foundation for future studies.

Our findings of increased circulating TIMP-3 concentration in PE are in agreement with previous reports describing hypomethylation of the *TIMP-3* promoter [21, 22], and increased *TIMP-3* expression in placental tissue from women with PE [21]. Noteworthy, our previous systematic review showed that DNA methylation is gene-specific or specific to genomic regions [24]. Similarly, by means of a sub-analysis of publicly available data deposited in Gene Expression Omnibus, we have recently shown that DNA hypomethylation is specific for the promoter region of the *TIMP-3* gene, leading to increased *TIMP-3* mRNA levels [20]. Moreover, the lack of differences on plasma TIMP-3 levels between early-onset PE and late-onset PE reinforce our earlier results regarding DNA methylation and gene expression showing that TIMP-3 is not able to differentiate between these two PE phenotypes [20].

Although the pathogenesis of PE is not entirely known, placental malperfusion leading to widespread maternal endothelial dysfunction is accepted as a major disease mechanism. Thus, set as two pathophysiological stages, abnormal placental formation occurs early in the first trimester followed by a maternal syndrome in the last two trimesters characterized by an excess of anti-angiogenic and pro-inflammatory factors [2]. We have previously suggested that the MMP-TIMP system may participate in both phases of PE [25]. Trophoblast invasion of spiral arteries and proper placentation require balanced expression levels of MMPs and TIMPs [17]. Indeed, imbalanced placental expression of MMPs and TIMPs and their plasma levels have been reported in hypertensive disorders of pregnancy [11, 12], including PE [13–18]. There is robust correlative and functional evidence indicating that TIMP3 is a key regulator of extracellular matrix remodeling during embryo implantation, as reviewed elsewhere [9]. Therefore, increased TIMP-3 levels could reduce MMP activity and lead to abnormal placental formation and function in PE. Meanwhile, our results showed significant positive correlations between TIMP-3 and MMP-2, which was surprising due to the inhibitory role of TIMPs on MMPs. However, the interaction between MMPs and TIMPs is very complex, being TIMPs also involved in activation and uptake/removal of MMPs from the extracellular environment [26]. For instance, the activation of pro-MMP-2 by matrix type 3-MMP was shown to be enhanced by TIMP-3 in a dose-dependent manner [27]. The interplay between TIMP-3 and TIMP-1 has yet to be determined by future studies.

Nonetheless, TIMP-3 has functions beyond the modulation of MMP activity, such as angiogenesis and apoptosis. It has been demonstrated that TIMP-3 is a strong inhibitor of the pro-angiogenic action of Vascular Endothelial Growth Factor (VEGF), through its binding to the VEGF type 2 receptor (VEGFR2) [28]. Alternatively, TIMP-3 may bind to angiotensin II (Ang II) type 2 receptor (ATR2), and the overexpression of both TIMP-3 and ATR2 additively inhibits VEGF-induced angiogenesis [29]. Moreover, TIMP-3 and ATR2 have also additive effects on inducing apoptosis through activation of different caspases [29]. Additionally, TIMP-3 is able to inhibit tumor necrosis factor-α converting enzyme, activating the apoptosis process in cultured rat vascular smooth muscle cells and tumor cell lines [30, 31]. Thus, TIMP-3 anti-angiogenic and pro-apoptotic properties may contribute

to abnormal placentation associated with PE. Future prospective studies in humans as well as studies in animal and cell models are warranted to confirm these roles of TIMP-3 in the pathophysiology of PE.

Furthermore, hypertension is marked by structural remodeling of vascular extracellular matrix and the balance between MMPs e TIMPs may be altered in response to a hypertensive stimulus [32]. It has been shown that Ang II-induced hypertension in significantly suppressed in mice lacking the *TIMP-3* gene, indicating a critical effect of TIMP-3 on preserving remodeling of the arterial extracellular matrix in response to Ang II [32]. Moreover, previous studies have demonstrated that MMP-2 is able to generate vasoconstrictors (e.g. endothelin-1-related peptides) and degrade vasodilators (e.g. adrenomedullin and calcitonin gene related-peptide) and cell surface receptors (e.g. VEGFR2 and β(2)-adrenergic receptor). Although unproven in the context of PE, MMP-2 may contribute to vasoconstriction and hypertension in PE through its extracellular matrix-independent actions [25]. Here we found an elevated TIMP-3 concentration in the circulation of PE and GH women. Similarly, higher circulating MMP-2 levels have been reported in PE compared to both HP and NP women [14, 35–37]. Therefore, future studies should investigate the role of TIMP-3 in stimulating MMP-2 activation and their isolated and/or interactive action on promoting vascular dysfunction and hypertension in PE.

As previous studies have demonstrated that TIMP-3 is the primary TIMP to regulate agonist-induced vascular remodeling and hypertension [32], we examined the relationship between TIMP-3 and blood pressure levels. Moreover, as obesity is an important risk factor for hypertension and PE [33], we have also determined the relationship between TIMP-3 and BMI. However, plasma TIMP-3 concentrations were not statistically associated with either blood pressure or BMI. Furthermore, TIMP-3 levels were not different between the PE and GH, nor between the early-onset PE and late-onset PE subgroups, suggesting that TIMP-3 is not a good blood-based biomarker to distinguish between hypertensive disorders of pregnancy or PE phenotypes. However, these analyzes might have been biased due to the fact that 1) the majority of PE and GH women were under anti-hypertensive therapy at the time of blood collection, and 2) patients with hypertensive disorders of pregnancy, especially PE, frequently develop edema, which influences BMI and hemodilution.

Noteworthy, we were able to validate our findings of increased circulating TIMP-3 concentrations in PE from the primary case-control study with the results in an independent population of pregnant women of the replication study. The subjects recruited for both studies were followed as outpatients in the Department of Gynecology and Obstetrics from their respective reference hospitals as soon as HP women entered the second trimester or when pregnant women were diagnosed with PE or GH through the gestation and postpartum. The recruitment protocols are described elsewhere [14, 38].

Although we have not enrolled patients with GH in the replication study, the main focus of this study in particular was to determine circulating TIMP-3 levels in an independent population of patients with PE. In this context, we were not able to retrieve all relevant clinical data for the patients with PE and HP women included in the replication study, as well as to measure MMP-2, MMP-9, TIMP-1 and TIMP-2 in their plasma.

In conclusion, our novel findings provide evidence for increased circulating TIMP-3 concentrations in patients with PE and GH compared with HP women. Moreover, increased circulating TIMP-3 concentrations were positively correlated with plasma MMP-2 and TIMP-1 concentrations in patients with PE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The authors confirm that data supporting the findings of this study are presented. Raw data are available from the corresponding author upon reasonable request.

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Synopsis:

Circulating TIMP-3 concentration is increased in pre-eclampsia compared to healthy pregnant women, and it is positively correlated to plasma MMP-2 and TIMP-1 concentrations in pre-eclampsia.

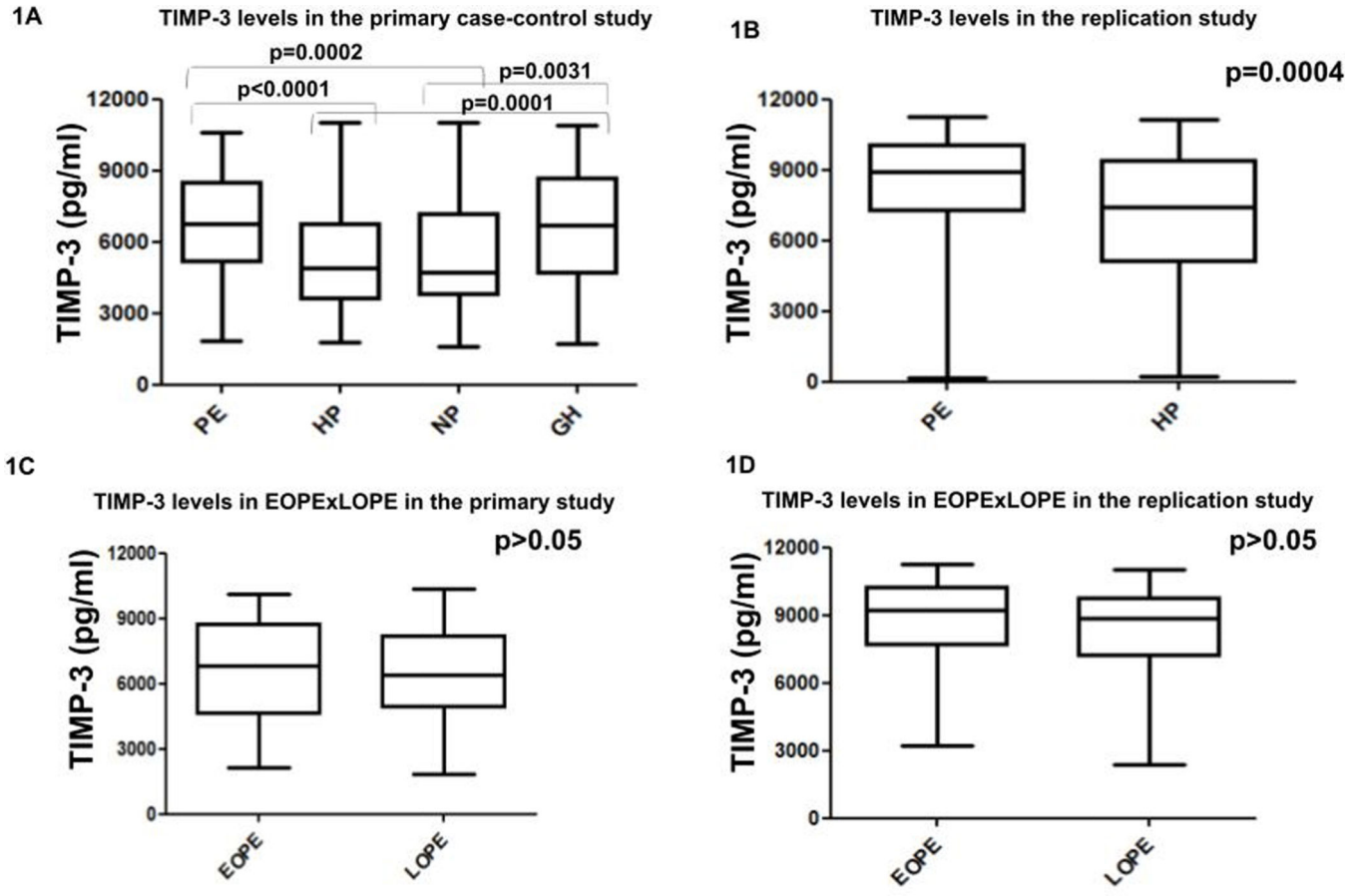

Figure 1.

Circulating TIMP-3 concentrations considering the women with pre-eclampsia (PE), healthy pregnant (HP), non-pregnant (NP) and gestational hypertension (GH), and into the subgroups of early-onset PE (EOPE) and late-onset PE (LOPE) in the (**A and C**) primary study and in the (**B and D**) replication study. The box and whiskers plots show range and quartiles. The boxes extend from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and lowest values.

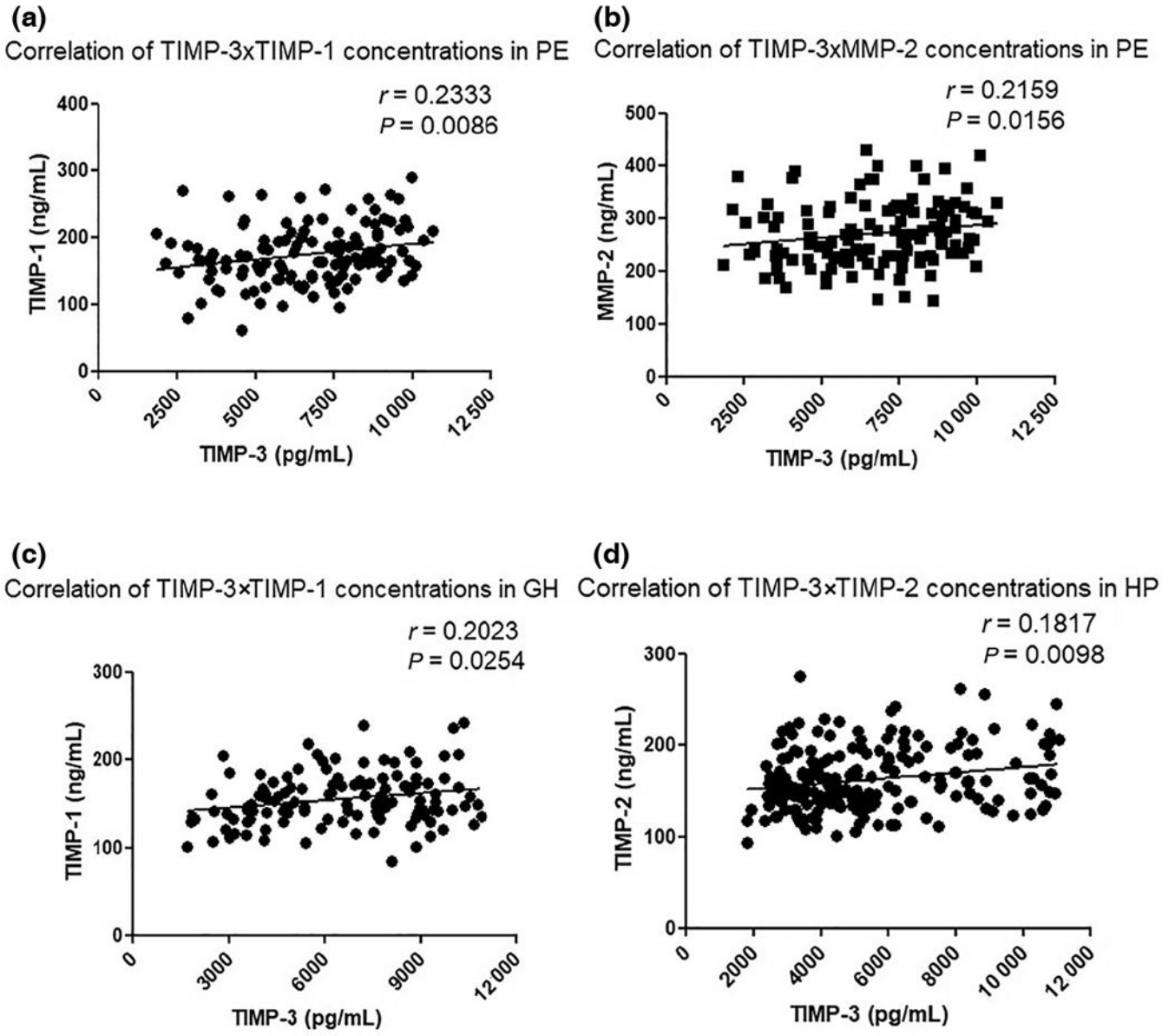

Figure 2.

Correlations between TIMP-3 concentrations with (**A**) TIMP-1 and (**B**) MMP-2 concentrations in women with pre-eclampsia (PE), and with (**C**) TIMP-1 and (**D**) TIMP-2 concentrations in women with gestational hypertension (GH) and healthy pregnant (HP) enrolled in the primary case-control study, respectively. The regression lines are plotted. The r- and p-values are reported. r: Spearman's correlation.

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Table 1.

Clinical, demographic and biochemical characteristics of women enrolled in the primary study.

Parameters	Pre-eclampsia (n=219)	Gestational hypertension (n=118)	Healthy pregnant (n=214)	Non-pregnant (n=66)	*P-value*
Age (years)	27.1±6.7	27.3±6.7	24.6±6.5	30.9±8.7	**0.0017**[a]; **0.0124**[b]; **0.0004**[c]; **0.0272**[d]
Ethnicity (% white)	68.2	70.3	71.03	77.14	
Current smokers (%)	9.04	12.7	7.36	5.45	
BMI (kg m-2)	33.2±6.6	35.6±7	33.2±6.6	22.7±2.1	0.9992[a]; **<0.0001**[b]; **0.0136**[c]; **<0.0001**[d]
SBP (mmHg)	138.3±18.6	132.1±18.1	110.7±11.3	114.3±9.6	all **<0.0001**[a,b,c,d]
DBP (mmHg)	86.9±12.9	84.2±12.5	81.8±10.4	77.4±6.8	**0.0013**[a]; **<0.0001**[b]; 0.1392[c]; **0.0002**[d]
HR (beats/min)	81.6±7.6	81.6±7.3	81.2±8.8	ND	0.9083[a]; 0.7850[c]
Fasting glucose (mg dl −1)	80.2±18.1	77.7±10	75.4±10.4	ND	0.1489[a]; 0.0968[c]
Hemoglobin (g dl −1)	11.7±1.5	11.9±1.3	11.9±1.5	ND	0.3693[a]; 0.3720
Hematocrit (%)	35.1±4.6	36±3.9	35.4±4.9	ND	0.6850[a]; 0.3449[c]
Creatinine (mmol l)	0.7±0.3	0,6±0.1	0.7±0.1	ND	0.4455[a]; **0.0113**[c]
24-h Pr (mg/24 h)	1465.1±2035.1	162.9±78.7	ND	ND	
GAD (weeks)	36.2±3.6	38.9±1.6	39.6±1.4	ND	**<0.0001**[a]; **0.0022**[c]
Newborn weight (g)	2617.3±919	3210.8±538.7	3271.1±510	ND	**<0.0001**[a]; 0.4166[c]
GAS (weeks)	34.1±4.1	35.4±4.9	36.6±3.3	ND	**<0.0001**[a]; 0.2338[c]
Early-onset PE (%)	20.9	ND	ND	ND	
Preterm birth (%)	16	ND	ND	ND	
IUGR (%)	9.9	ND	ND	ND	
TIMP-3 (ng/mL)	6764.9±2136.1	6581.4±2425.7	5444.3±2400.1	5478.8±2556.9	**0.0001**[a]; **0.0002**[b]; **0.0001**[c]; **0.0031**[d]

Abbreviations: BMI, Body mass index; DBP, Diastolic blood pressure; GAD, Gestational age at delivery; GAS, Gestational age at sampling; HR, Heart rate; IUGR, Intrauterine growth restriction; ND, Not determined (however, negative dipstick test); PE, Pre-eclampsia; Pr, Proteinuria; SBP, Systolic blood pressure; SEM, Standard error of the mean; TIMP-3, Tissue inhibitor of matrix metalloproteinase-3. Values are the mean ± SEM. Bold values are significant (<0.05), according to the comparisons between groups:

[a] p<0.05, Pre-eclampsia *versus* healthy pregnant women;

[b] p<0.05, Pre-eclampsia *versus* non-pregnant women;

[c] p<0.05, Gestational hypertension *versus* healthy pregnant women;

[d] p<0.05, Gestational hypertension *versus* non-pregnant women.

Table 2.

Clinical, demographic and biochemical characteristics of women enrolled in the replication study

Parameters	Healthy pregnant (*n* = 124)	Pre-eclampsia (*n* = 177)	*P* value
Age, y	26.72±0.710	27.32±0.5828	0.5177
Ethnicity % white	84	89	-
GAS, wk	34.33±0.3538	31.87±0.526	**0.0001**
Parity			
Nulliparous	64	68	-
Multiparous	36	32	-
SBP, mm Hg	115.7 ±0.7	155.8±0.2	**<0.0001**
DBP, mm Hg	73.3 ±0.1	103.3±0.2	**<0.0001**
24-h Pr, mg per 24 h	ND	1857 ±539.4	-
Uric acid, mg/dl	ND	5.15±0.2	-
Early-onset PE	ND	41	-
Severity			
Severe PE	ND	57	-
Mild PE	ND	43	-
TIMP-3, pg/ml	7296±223.8	8349 ±162.4	**0.0004**

Abbreviations: GAS, gestational age at sampling; SBP, systolic blood pressure; DBP, diastolic blood pressure; 24-h Pr, 24-h proteinuria; ND, not determined; SEM, Standard error of the mean; TIMP-3, Tissue inhibitor of matrix metalloproteinase-3.

Values are the mean ± S.E.M. Bold values are significant according to the comparison:

* p<0.05, Pre-eclampsia *versus* healthy pregnant women.