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The impact of family history of preeclampsia and alteration of MMP-13/TIMP-1 balance in the occurrence of preeclampsia

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Abstract

Tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) suppresses the activity of matrix metalloproteinase-13 (MMP-13). The MMP-13/TIMP-1 complex has been proposed as one of the collagen types I and III turnover regulators in a healthy pregnancy. This study intends to investigate the distribution of serum levels of MMP-13, TIMP-1 and the ratio of MMP-13/TIMP-1 in women with preeclampsia (PE) with a family history of preeclampsia. We examined 37 patients with PE, with a mean age of 24.9±6 years, while the mean age of the control group of 32 healthy subjects was 24.7±5.4 years. We divided patients into two subgroups: subjects with a family history of PE (n=18); (PE+FHPE) and subjects without a family history of PE (n=19); (PE-FHPE). We measured MMP-13 and TIMP-1 levels via enzyme-linked immunosorbent assay (ELISA), and calculated the ratio. Either MMP-13 or TIMP-1 alone did not exhibit any obvious differences between normal and PE pregnancies. MMP-13/TIMP-1 ratio was statistically significantly higher in PE than healthy pregnant women: 0.2 (0.1÷0.5) vs. 0.065 (0.05÷0.2) ($p<0.05$). Patients with PE+FHPE showed statistically significantly lower MMP-13/TIMP-1 ratio compared with PE-FHPE 0.085 (0.05÷0.25) vs. 0.22 (0.12÷0.35) (KW=5.71; p=0.02). These results indicate an altered balance between MMP-13 and TIMP-1 in PE patients with a family history of preeclampsia. Further studies with more precise analysis and genetic methods are needed to elucidate how the imbalance between MMP-13 and TIMP-1 contributes to PE susceptibility and pathogenic mechanisms determining preeclampsia development.

Keywords: matrix metalloproteinase-13, tissue inhibitor of matrix metalloproteinase-1, family history of preeclampsia, collagen types I and III

1. Introduction

Preeclampsia (PE) is a hypertensive disorder of pregnancy, defined by the occurrence of new-onset hypertension (140/90 mmHg) and either proteinuria (0.3g in a 24h urine sample) or end-organ dysfunction developing after 20 weeks of gestation (1). It is a major cause of maternal and perinatal morbidity and mortality (2, 3). However, it has not been thoroughly explored.

The two major proteins of the human uterine wall are collagen type I and type III. In a healthy pregnancy, collagen metabolism increases as the uterus develops. Preeclampsia has been associated with altered collagen turnover leading to impaired modification of the uterine wall structure. Type I and III collagen play a central role in this abnormal process (4, 5). Therefore, it is proposed that these fibrillar proteins' turnover (the primary function of which in normal pregnancy is to maintain the uterine consistency and support the scaffold's stability) could be pathologically affected.

Matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) are essential regulators of extracellular matrix (ECM) remodeling. It is well known that dysregulation of the MMP/TIMP complex expression/activity leads to structural collagen damage (6, 7). During pregnancy, abnormally expressed MMPs have been reported to cause hypertensive disorders. In preeclampsia, these mechanisms have also been assumed to play a key role in altered uterine and vascular ECM turnover characterized by abnormal vasodilation, placentation, and the development of generalized vascular damage (8, 9). MMP-13 breaks down ECM proteins such as collagens and fibronectins. It degrades "triple helical collagens, including type I, II, and III collagens, but has the highest soluble type II collagen activity. It can also degrade

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type IV, type XIV, and type X collagens and play a role in wound healing and tissue remodeling, in cell migration and tumor cell invasion" (10). MMP-13 plays a vital role in tissue regeneration and the pathogenic mechanisms of certain diseases such as atherosclerosis, aneurysms, and cancer. In reference to TIMP-1, it is a glycoprotein. This biomolecule is a crucial part of the TIMPs family (11).

Several tissues have been described to express TIMP-1 (12). The primary function of this protein is to inhibit the activity of matrix metalloproteinases, thus suppressing ECM degradation (13). With the accumulation of additional knowledge about the structure and function of TIMP-1, it was revealed that it potently inhibits the activity of most MMPs except MMP-2 and MT1-MMP (14). Hence, it can be theorized that TIMP-1 plays a role as an inhibitor of MMP-13.

It can be concluded that failure of the regulation of MMPs/TIMPs complex occurs in preeclampsia. This leads to pathological collagen I and III turnover and abnormal uterine wall remodeling, which results in impaired modification of uterine wall collagen structure. It has been postulated that control of expression and regulation of MMP-13 and TIMP-1 might be crucial in complicated pregnancy. However, there are no data in the literature on a parallel examination of MMP-13 and TIMP-1 serum concentrations and their ratio in preeclamptic women with a family history of preeclampsia.

The present study aimed to investigate the distribution of serum levels of MMP-13, TIMP-1 and the ratio of MMP-13/TIMP-1 in women with preeclampsia with a family history of preeclampsia.

2. Material and methods

2.1. Study population

The current research was a cross-sectional study and part of a university scientific project (N1/2020). The Ethics Committee of the Medical University- Pleven approved the project with Protocol N51/2020. All participants signed informed consent. Study procedures followed all guidelines for ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975, as revised in 2000.

All participants were in-patients of the Clinic of Obstetrics and Gynecology, University Hospital "G. Stranski" Pleven. The sera of subjects were taken from October 2019 to March 2021. The study group consisted of 37 women with preeclampsia, with a mean age of 24.9±6 years, while the mean age of the control group of 32 women with normal pregnancies was 24.7±5.4 years. We divided the patients into two subgroups: Subjects with a family history of PE (n=18); (PE+FHPE) and subjects without a family history of PE (n=19); (PE-FHPE).

Criteria for inclusion in the study were as follows: Pregnant women with clinical symptoms and laboratory criteria for preeclampsia (We used the 2018 European Society of Cardiology Guideline for the management of cardiovascular diseases during pregnancy for the diagnostic criteria of preeclampsia); gestational hypertension with significant proteinuria (>300mg/24h urine collection or the extrapolated amount from a timed collection) (15); maintaining a current diet and exercise during the study; signed informed consent to participate in the study; dysfunction of mother's organ such as HELLP syndrome, renal failure, neurological involvement, hepatic involvement or fetal growth retardation. Criteria for exclusion from the study were as follows: diabetes mellitus, kidney and heart disease, signs of chorioamnionitis, and the presence of a fetus with a chromosomal abnormality.

2.2. ELISA

We used ELISA to determine MMP-13 and TIMP-1 and measured MMP-13 and TIMP-1 levels in serum samples using ELISA kits (Human MMP-13 ELISA kit Reagent Genie; Human TIMP-1 Quantikine ELISA kit) according to the manufacturer's instructions. We calculated the MMP-13/TIMP-1 ratio.

2.3. Statistical analysis

We used the following computer programs to analyze the research data: Excel (Microsoft Corporation, Redmond, WA), SPSS and Statgraphics Plus (Manugistics, Rockville, MD) for Windows. We used tables, graphs, and numerical values to describe all results. We determined the level of significance as $p<0.05$. We used standard skewness and kurtosis to check the normality of distribution and equality of variances. We used student's t-test and ANOVA with mean±SD in cases with normal distribution to find significant differences between groups (LSD, Tukey HSD, Scheffe, Bonferroni, Newman-Keuls, Duncan). We used □2 and K-W H-tests with a median (M) value in cases without normal distribution, together with first and third quartile Q1 and Q3; (twenty-fifth and seventy-fifth percentile P25 and 75P).

3. Results

Either MMP-13 or TIMP-1 alone did not exhibit any obvious differences between normal and PE pregnancies (Table 1) (Fig. 1 and 2). However, MMP-13/TIMP-1 ratio was statistically significantly higher in PE than healthy pregnant women: 0.2 (0.1÷0.5) vs. 0.065 (0.05÷0.2) ($p<0.05$) (Fig. 3). Moreover, patients with PE+FHPE showed statistically significantly lower MMP-13/TIMP-1 ratio compared to PE-FHPE 0.085 (0.05÷0.25) vs. 0.22 (0.12÷0.35) (KW=5.71; p=0.02) (Fig. 4).

Table 1. Serum levels of MMP-13 and TIMP-1 in healthy pregnant women and preeclampsia

	Healthy pregnant women	Preeclampsia	P
MMP-13 (ng/ml)	0.17 (0.15÷0.2)	0.18 (0.16÷0.2)	p>0.05
TIMP-1 (ng/ml)	3.02 (1.28÷3.58)	2.41 (1.01÷4)	p>0.05

MMP-13: matrix metalloproteinase 13, TIMP-1: tissue inhibitor of matrix metalloproteinase 1; Data are expressed as median (interquartile range)

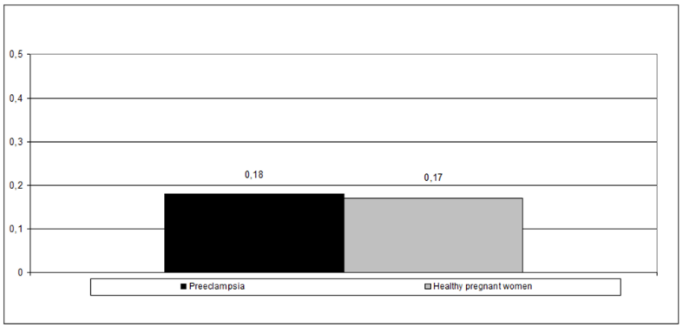

Fig. 1. Serum levels of MMP-13 (ng/ml) in women with preeclampsia vs healthy pregnant women; MMP-13: matrix metalloproteinase 13; Data are expressed as median

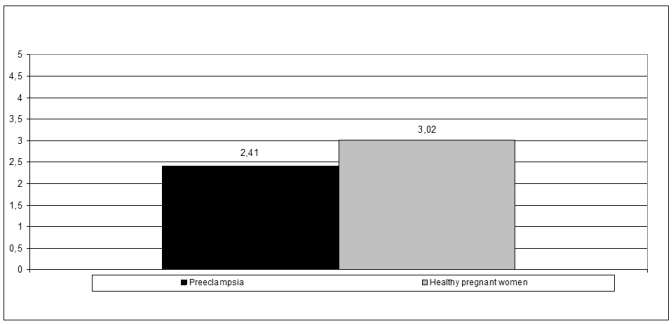

Fig. 2. Serum levels of TIMP-1 (ng/ml) in women with preeclampsia vs healthy pregnant women; TIMP-1: tissue inhibitor of matrix metalloproteinase 1; Data are expressed as median

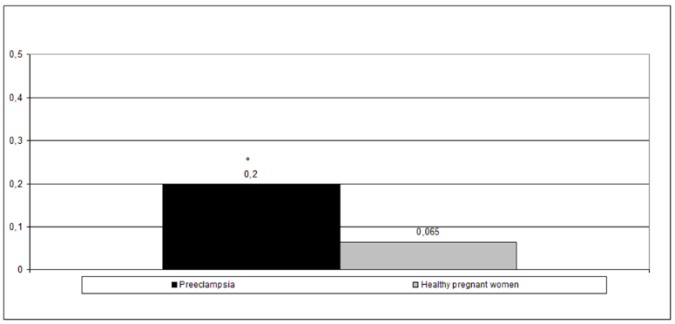

Fig. 3. MMP-13/TIMP-1 serum ratio in women with preeclampsia vs healthy pregnancy; MMP-13/TIMP-1 ratio was significantly higher in preeclampsia than in healthy pregnant women: 0.2 vs 0.065 (*p<0.05); Data are expressed as median

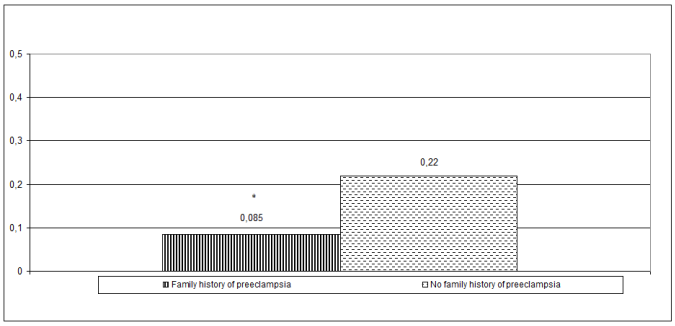

Fig. 4. MMP-13/TIMP-1 serum ratio in women with preeclampsia according to the presence or absence of a family history of preeclampsia; Patients with a family history of preeclampsia showed a significantly lower MMP-13/TIMP-1 ratio than subjects without a family history: 0.085 vs 0.22 (p=0.02); Data are expressed as median

4. Discussion

Preeclampsia is a vital pregnancy complication and one of the most common pregnancy disorders. Preeclampsia is characterized by a mother's high blood pressure, often with proteinuria. Fetal growth restriction is also generally observed. Early preeclampsia detection is paramount for risk stratification and preventing further complications. There is evidence that uterine ECM metabolism is altered during preeclampsia. However, data in the medical literature about the modifications of collagen in the human uterus is limited (16, 17). The courses of collagen types I and III synthesis and degradation in the uterine ECM are "dynamic and reflect healthy and complicated pregnancy" (18). Accumulating data proves that collagen type I and III turnover is impaired in preeclampsia. This might be a consequence of disrupted uterine ECM homeostasis and MMP/TIMP system imbalance (19).

Considering the conception of MMPs and TIMPs' crucial role in the pathogenic pathways in preeclampsia, Karthikeyan et al. (20) studied these proteins and reported that plasma and genetic alterations in the MMP/TIMP system are associated with hypertensive disorders of pregnancy. Authors have found that if the regulation of the MMP/TIMP system fails in the control of the ECM remodeling, this may lead to diseases such as gestational hypertension and preeclampsia. Furthermore, vascular "remodeling disorders of the uterine and placenta and placenta hypoperfusion have been generally recognized" (21). The following literature data represents current studies exploring levels of MMP-13 and TIMP-1 in samples of patients with hypertensive disorders of pregnancy:

In 2017, Laskowska tested the hypothesis of whether maternal serum MMP- 2, 3, 9, and 13 levels have different values in early- and late-onset preeclampsia and uncomplicated pregnancies. “The levels of MMP-13 were higher in both preeclamptic groups of pregnant women than in the healthy controls, but these differences were statistically insignificant. A critical finding of the present study was that MMP-3 appears to be involved solely in early-onset preeclampsia but not in late-onset preeclampsia. Higher levels of MMP-2 and MMP-13 and lower levels of MMP-9 seem to be related to both early- and late-onset severe preeclampsia" (22). Using ELISA, Tayebjee et al. (2005) investigated TIMP-1 and -2 levels in women with gestational hypertension, normotensive women with normal pregnancies and healthy non-pregnant control subjects. Levels of TIMP-1 and TIMP-2 were significantly different among the three groups. Authors concluded that "altered MMP/TIMP ratios in maternal blood during gestational hypertension. These observations suggest pregnancy-related changes in ECM breakdown and turnover. Given the importance of changes in ECM composition to vascular and cardiac structure in hypertension, investigators suggest that these observations may be related to the pathophysiology of human gestational hypertension" (23).

Luizon et al. (2014) examined TIMP-1 polymorphism, plasma TIMP-1 levels, and antihypertensive therapy responsiveness in hypertensive disorders of pregnancy. They measured plasma MMP-9 and TIMP-1 levels using ELISA. Gestational hypertension patients with the GG genotype for the TIMP-1 polymorphism had lower MMP-9 levels and MMP-9/TIMP-1 ratios than those with the TT genotype. Preeclampsia patients with the TG genotype had higher TIMP-1 levels (24).

Gupta M et al., 2016, explored the serum concentrations of MMP-1, TIMP-1 and their ratio in the second and third trimesters of normal and preeclamptic pregnancy. The investigators examined these biomolecules via the ELISA method. Serum levels of MMP-1, TIMP-1, and their ratio during the progression of preeclampsia did not show statistical significance compared to normal pregnancy (25). Myers JE et al.'s (2005) investigation involved women who subsequently developed preeclampsia. Authors studied MMP-2 and-9 levels, TIMP-1 and TIMP-2 in the plasma of this contingent of patients. Plasma samples were taken from women whose pregnancies were subsequently complicated by preeclampsia and from normal pregnant women at 22 and 26 weeks and delivery or diagnosis. "Following equal protein loading, MMP-2 and 9 and TIMP-1 and 2 were quantified using zymography and Western blot analysis, respectively. TIMP-1 levels were significantly reduced in the preeclampsia group at 26 weeks ($p = 0.0002$), but TIMP-2 levels were not quantifiable. Authors conclude that at all three gestational time points an imbalance in the MMP-2/TIMP-1 ratio was found in patients who subsequently developed preeclampsia" (26). In their research, Palei et al., 2008, focused on patients with preeclampsia and gestational hypertension and compared their data with those of normotensive pregnancies and healthy non-pregnant women. The investigators examined the concentrations of TIMP-1 and TIMP-2 in the subjects mentioned above. TIMP concentrations were measured in plasma samples by gelatin zymography and ELISA. The results of Palei et al. showed higher TIMP-1 levels in PE than in GH and normotensive pregnant women (27). In another study, Ab Hamid et al. (2012) determined the total levels of TIMP-1and 2 by ELISA. The contingent taking part in the study involved women with gestational hypertension and normotensive pregnant women. The expression levels of TIMP-1 and TIMP-2 in the gestational hypertension group were low (28).

In 2014, forty-seven biomarkers involved in the pathogenesis of preeclampsia were determined in 5623 pregnant women, part of a prospective investigation named "Early Pregnancy Prediction of Preeclampsia in Nulliparous Women, Combining ClinicalRisk and Biomarkers the Screening for Pregnancy Endpoints (SCOPE) International Cohort Study ". These biomolecules' levels were surveyed in the plasma of subjects sampled at 14 to 16 weeks gestation. Preeclampsia developed in 278 women, approximately 5% of all subjects participating in the study. TIMP-1 showed higher levels in these patients than in the no preeclampsia group (29). In order to estimate the role of MMP-2 and -9, along with their inhibitors TIMP-1 and-2, Montagnana et al. (2009) evaluated these indicators using ELISA in preeclamptic, normotensive pregnant and non-pregnant women. The serum levels of TIMP-1 were significantly higher in preeclamptic compared to both non-pregnant and normotensive pregnant women (30).

In our study, serum MMP-13 levels in preeclampsia were higher than in women with normal pregnancies, but not significantly. Current findings are consistent with the study of Laskowska mentioned above (22). As for TIMP-1, patients' levels were insignificantly lower than healthy pregnant women. Our results are in line with those of Gupta et al. (25) but in a contradiction with the reports of the researchers Luizon et al. (24), Montegrana et al. (30), as well as Paley and co-authors (27). A possible explanation for the lack of a TIMP-1 significant difference in our investigation was the likely predominance of different TIMP-1 polymorphisms in which TIMP-1 levels did not increase significantly (24). In addition, there are also options for some differences in the sample types used between the different studies [plasma (23, 24, 26, 27, 29) vs. serum (25, 28, 30)], methods [ELISA (23-25, 27, 28, 30) vs. gelatin zymography (26, 27)], difference in the examined contingent of patients [gestational hypertension (23, 24, 27, 28) or preeclampsia (24-27, 29, 30)].

In the light of our observations, we continued our investigation and further analyzed the ratio of MMP-13/TIMP-1 between the study groups. In this context, we reported two compelling pieces of evidence: (1) significantly higher serum MMP-13/TIMP-1 ratio in preeclampsia than in normal pregnancy subjects and (2) significantly lower MMP-13/TIMP-1 ratio in PE+FHPE compared to PE-FHPE. Given the present results, a question of great interest arises. So, what could be the reason favoring patients with a history of preeclampsia to indicate a lower MMP-13/TIMP-1 ratio than subjects without a family history? The following theory might possibly explain our findings. We hypothesize that in preeclampsia, the precise balance between the degradation activity of collagenase MMP-13 and tissue inhibitor TIMP-1 is disturbed. As a result of this dysregulation, collagen type I and III turnover are impaired. This may favor abnormal vascular and uterine ECM changes at the maternal-fetal interface. These processes lead to the over deposition of collagen, which may affect uterine remodeling. It should be considered that we just assessed the levels of circulating MMP-13, TIMP-1 and their ratios, so the role of these components' expression/activity cannot be commented upon. Of note, we closely monitored the subgroup of patients with a history of preeclampsia during the whole investigation, also following them post-study. We marked their pregnancy outcomes, possible later hypertension development, and other results. We are still analyzing all these data to be used for future publications.

The major finding of the present study was the disrupted

balance between MMP-13 and TIMP-1 in preeclamptic women with a family history of preeclampsia. Hereby, we suggest that altered steadiness between collagen type I and III synthesis and degradation might play an essential role in PE susceptibility and the development of preeclampsia. To the best of our knowledge, our study has provided for the first time the equilibrium between serum MMP-13 and TIMP-1 in preeclamptic women with a family history of preeclampsia.

Our study revealed an altered balance between the MMP-13 and TIMP-1 in PE patients with a family history of preeclampsia. The net effect of this imbalance might contribute to the development and progression of PE, but the exact mechanisms are yet unclear. The small sample size and the cross-sectional design were limitations of the current study. More extensive and longitudinal studies, including more sensitive methods like Western blot, gelatin zymography and analysis of the genes encoding MMP-13 and the protein segment of TIMP-1 are needed to explore in detail the clinical significance of MMP-13/TIMP-1 complex in preeclampsia.

Our findings provided evidence for diminished serum MMP-13/TIMP-1 ratio in preeclamptic women with a family history of preeclampsia. The imbalance between MMP-13 and TIMP-1 could be a factor contributing to the pathogenic mechanisms determining susceptibility and development of preeclampsia.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.N., N.P., Design: A.N., N.P., Data Collection or Processing: N.P., Analysis or Interpretation: A.N., N.P., Literature Search: N.P., I.H., Writing: A.N., N.P.

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