ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 28(2): 307-313, 2023
DOI: 10.5505/ejm.2023.46872

Investigation of Matrix Metalloproteinas Levels In Preeclamptic/Eclamptic Pregnancy

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ABSTRACT

The present research was designed to investigate levels of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), and their respective tissue inhibitors, tissue matrix metalloproteinase inhibitor-1 (TIMP-1) and tissue matrix metalloproteinase inhibitor-2 (TIMP-2), in eclamptic and preeclamptic pregnant women.

In this work, undertaken in the Obstetrics and Gynecology Department of the Yüzüncü Yıl University Faculty of Medicine Health Practice and Research Hospital, eclamptic (n=28), preeclamptic (n=28), and healthy pregnant women (n=24) were enrolled and their levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 were studied by micro-ELISA method.

No statistically meaningful differences were identified between the study groups for MMP-2 (p=0.786). When the ratios of MMP-2 to TIMP-2 of the three groups were examined, again, no statistically meaningful differences were noted (p=0.788). While a meaningful difference was not seen between the preeclamptic and eclamptic groups for MMP-9 and TIMP-1, significantly lower values of MMP-9 and TIMP-1 were obtained in both groups upon comparison to the control group (p=0.001 for MMP-9, p<0.000 for TIMP-1). When the MMP-9/TIMP-1 ratios of the groups were evaluated, a statistically meaningful difference did not exist between women with preeclampsia and controls; however, this ratio was higher among women with eclampsia in comparison to the preeclampsia and control groups and this finding was statistically significantly (p=0.011).

Imbalanced levels of MMP-9 and TIMP-1 could be used as a marker in efforts to predict preeclampsia and especially eclampsia. However, larger clinical studies are needed to reveal these interactions in more detail.

Keywords: Matrix metalloproteinase, preeclampsia, eclampsia, pregnancy

Introduction

Hypertension in pregnancy may be described as a multisystemic disease causing serious fetal and maternal mortality and morbidity, and its pathophysiology is not fully understood. Preeclampsia is defined as a multisystem progressive disorder characterized by the new onset of hypertension (Systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) and proteinuria or the new onset of hypertension and significant end-organ dysfunction with or without proteinuria after 20th week of pregnancy or postpartum period (1).

Eclampsia entails the addition of convulsions to preeclampsia. Roughly 5-10% of all pregnancies are complicated by hypertension (2, 3). Although there are many accepted theories for it, the pathophysiology of preeclampsia is still not fully understood. The placental functioning needed for a successful pregnancy depends on adequate trophoblastic invasion, and the point emphasized most in efforts to understand preeclampsia is the lack of sufficient invasion by trophoblasts (4). In a healthy placenta, extravillous trophoblasts enter the elastic lamina and layers of the smooth middle muscle of the mother's spiral arteries (5), a phase that ends by the 20th week of pregnancy. With this process, the sizes of uterine spiral arteries are increased while the vasoconstrictive ability is decreased and they are transformed into vessels with high rates of flow and low resistance (6). Such morphological processes have evolved to ensure augmented placental perfusion (7).

By anticipating patients who may develop preeclampsia and making an early diagnosis, effective treatment can be applied to this patient group to reduce maternal morbidity and mortality. In order to develop new diagnostic methods, it is necessary to understand the pathophysiological causes of the disease and to discover new diagnostic methods. Markers have been sought for

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Received: 10.02.2022, **Accepted:** 02.07.2022

the purpose of identifying pregnant women who may develop preeclampsia. Despite many studies to date, however, a predictor with reliable levels of both specificity and sensitivity for the development of the disease has not yet been identified.

The extracellular matrix (ECM), an interactive and dynamic structure, creates a special environment in intercellular spaces (8). It helps to hold cells in tissues together and also acts as a reservoir for many hormones that control cell growth and differentiation (10). As the cells and the matrix interact, the process is controlled by proteolytic enzymes (extracellular proteases), compounds that direct the hydrolysis of the components of the ECM. Matrix metalloproteinases (MMPs) constitute an important group among these enzyme systems.

Reduced activities of MMPs have been suggested to trigger failure in the processes of trophoblastic invasion of maternal spiral arteries, subsequently creating a decrease in the levels of fetoplacental perfusion with excretion of placental substances that adversely impact the vascular tone and structural regulation (10). The present work was planned with the aim of evaluating the potential use of MMP-2, MMP-9, and their respective tissue inhibitors, tissue matrix metalloproteinase inhibitor-1 (TIMP-1) and tissue matrix metalloproteinase inhibitor-2 (TIMP-2), as potential predictive biochemical markers for preeclampsia/eclampsia by evaluating them separately in the sera of preeclamptic/eclamptic and normotensive pregnant women.

General Information

MMPS and Tissue Mmp Inhibitors: The ECM is a dynamic and interactive structure that creates a special medium in intercellular spaces (8). It helps hold cells together in tissues while also acting as a reservoir for hormones that control cell growth and differentiation. It enables cells to interact directly or indirectly with intracellular signaling pathways that will direct them to perform special functions (9). These interactions between the ECM and cells have critical importance in the normal development and functioning of organisms (11).

Interactions of cells with the matrix are controlled by proteolytic enzymes, or extracellular proteases, compounds involved in the process of the hydrolysis of ECM components. These compounds have fundamental roles in controlling signals generated by matrix molecules, including involvement in cell differentiation, proliferation, and death, as a result of their impact on both the integrity and organization of the structure of the ECM (11, 12). The multifunctionality of extracellular proteases makes them potential therapeutic targets (12).

MMPs represent an important group among these enzyme systems. They constitute a family of zinc (Zn^{++})- and calcium (Ca^{++})-dependent neutral endopeptidases possessing the capability of degrading ECM components. A wide array of cells excrete these compounds, including endothelial cells, macrophages, vascular smooth muscle cells, T lymphocytes, platelets, chondrocytes, keratinocytes, epithelial cells, mesenchymal cells, neutrophils, trophoblasts, and osteoblasts (13, 14).

To ensure the ongoing continuation of fundamental physiological events for any organism, a consistent balance of MMP activities and those of their corresponding endogenous tissue inhibitors (TIMPs) is necessary. These two related groups of compounds are expressed in healthy tissues at low levels and take part in a wide variety of biological events. These include bone remodeling, angiogenesis, apoptosis, wound healing, inflammation, development of immune responses, ovulation, embryonic development, organ morphogenesis, nerve cell development, cervical dilatation, the endometrial cycle, and the hair follicle cycle, among others (14).

Materials and Methods

The present research was undertaken upon receiving the approval of the Yüzüncü Yıl University Faculty of Medicine's Ethics Committee between January 1 and October 20, 2009, in the Gynecology and Obstetrics Clinic of the Yüzüncü Yıl University Health Practice and Research Hospital (Ethics Committee number:2009/04). The women who participated in this research received detailed information about the study and provided both written and verbal consent before being enrolled.

Patients who were admitted to the clinic at 35-40 gestational weeks having been diagnosed with preeclampsia or eclampsia and who were not in active labor were eligible for enrollment in this work. Cases of high systolic blood pressure (≥140 mmHg) and/or diastolic blood pressure (≥90 mmHg) in conjunction with high levels of protein excretion (≥300 mg) in 24-h urine tests were accepted as cases of preeclampsia. Patients who met the criteria for preeclampsia and had also experienced at least one clonic seizure were considered to have eclampsia. Women with a history of gestational diabetes mellitus, premature

membrane rupturing, preterm labor, multiple pregnancy, polyhydramnios, chronic hypertension, or renal and hepatic disease were excluded. Patients who applied to the obstetrics and gynecology outpatient clinic for control appointments at 35-40 gestational weeks and who did not have any obstetric or other medical complaints were taken as the control group. A total of 80 patients (eclampsia = 28, preeclampsia = 28, normal pregnancy = 24) fulfilling the relevant inclusion criteria constituted the study population.

Data for all participating patients, including those in the control group, were recorded on research forms prepared beforehand. With these forms, the following information was recorded: patient's age, gravidity, parity, number of living children, pregnancy losses, height, weight, systolic and diastolic blood pressure, average blood pressure, gestational weeks (according to last menstrual period and ultrasound), number of seizures if eclamptic, hemoglobin, hematocrit, platelet count, biochemical values (blood urea nitrogen [BUN], urea, creatinine, uric acid, direct bilirubin, indirect bilirubin, glucose, aspartate transaminase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH], sodium, potassium, and calcium), and proteinuria values in spot urine and 24-h urine. After blood samples were taken from all participants and centrifuged at 4000 rpm for 10 minutes (Nucleus NF 00R, Ankara, Turkey), the obtained sera were stored in Eppendorf tubes at -80 °C. Serum samples were subsequently studied with an appropriate device in accordance with the directions of the manufacturer (DAS srl, Palombara Sabina, Italy) together with appropriate RayBio® Human MMP-9, MMP-2, TIMP-1, and TIMP-2 kits for enzyme-linked immunosorbent assay (ELISA; RayBiotech Life, Inc., Peachtree Corners, GA, USA) in the Department of Microbiology of Yüzüncü Yıl University. The levels of these four compounds were determined in the reading stage with a fully automatic Tri Turus device (Spain).

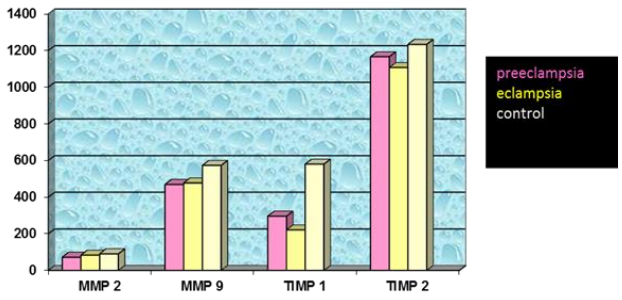
Statistical analysis was performed after transferring the patients' data to SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). In the following text, descriptive statistics for continuous variables are expressed as averages, standard deviations, and minimum and maximum values. For categorical variables, numbers and percentages are presented. Student's t-test was employed for the comparisons of group means for continuous variables, while Pearson's correlation coefficient was utilized with the aim of understanding interactions existing among these variables. On the other hand, chi-square tests were applied to confirm the presence of interactions between study groups and the considered categorical variables. In these evaluations, values of $p<0.05$ were accepted as significant.

Result

Eighty individuals, including preeclamptic (n=28), eclamptic (N=28), and healthy (n=24) patients of similar ages, were included in the population of this study performed in the Gynecology and Obstetrics Clinic of the Yüzüncü Yıl University Health Practice and Research Hospital. Statistically significant differences were not observed among these groups for age, parity, abortion ($p=0.226$), number of children who were born and died ($p=0.509$), number of stillborn children ($p=0.369$), number of living children, pulse, height, weight, hemoglobin, hematocrit, white blood cell count, fasting blood glucose, or BUN ($p>0.05$). Furthermore, a statistically meaningful difference was not obtained for gravidity between the preeclampsia group and either the eclampsia or the control group. However, gravidity was lower in the control group in comparison to the eclampsia group and this finding was significant ($p=0.037$).

Significant differences were not identified between the study participants with preeclampsia and eclampsia for systolic or diastolic blood pressure. When the participants with preeclampsia and eclampsia were considered in comparisons to the control group, however, statistically significantly higher values were observed in the former two groups compared to control participants (systolic and diastolic blood pressure: $p<0.001$ and $p<0.001$).

A meaningful difference was not determined between individuals with preeclampsia and eclampsia for protein in spot urine. When the women with eclampsia and preeclampsia were compared to healthy control participants, however, their protein levels were seen to be higher than those of the control participants and this finding was significant ($p<0.001$). Protein values obtained for 24-h urine were higher among preeclamptic women than eclamptic women and this finding was significant. When the preeclampsia and eclampsia groups were compared to the control group, once again, protein levels were seen to be higher in both of them than among control participants with statistical significance ($p<0.001$).

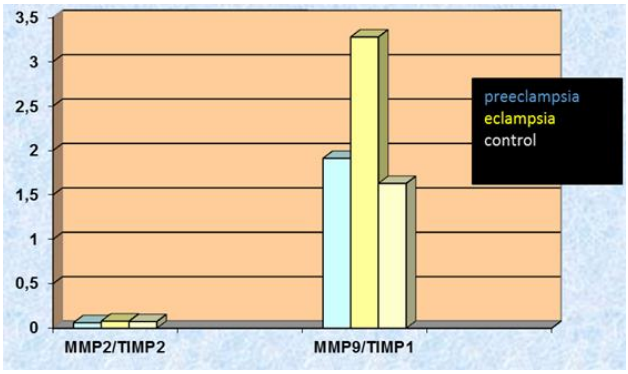


Graph 1. Graphical Portrayal of MMP and TIMP Levels of the Study Groups
Preekl.: Preeclampsia group; Eklampsi: Eclampsia group; Kontrol: Control group; MMP 2: Matrix metalloproteinase-2; MMP 9: Matrix metalloproteinase-9; TIMP 1: Tissue matrix metalloproteinase inhibitor-1; TIMP 2: Tissue matrix metalloproteinase inhibitor-2

In terms of platelet levels, no differences with statistical significance were observed between preeclamptic participants, eclamptic participants, and the women in the control group. However, platelet levels were lower among eclamptic participants than in the controls with statistical significance (p=0.049).

Upon comparing urea levels, no statistically meaningful difference was seen between the participants with eclampsia and preeclampsia. However, urea was found at significantly higher levels in the eclampsia and preeclampsia groups upon comparison to the healthy controls (p=0.002). For creatinine, no statistically meaningful difference was seen between the participants with eclampsia and preeclampsia. On the other hand, creatinine levels were seen to be elevated in the eclampsia and preeclampsia groups in comparison to the healthy control group and this finding was significant (p=0.027). In terms of uric acid levels, once again, a meaningful difference was not observed between participants with eclampsia and those with preeclampsia. Uric acid levels were found to be significantly higher in the eclampsia and preeclampsia groups upon comparison to the control group (p<0.001).

For AST levels, no statistically meaningful difference was obtained between participants with preeclampsia and the healthy women in the control group. Levels of AST measured in the eclampsia group, however, were seen to be higher in comparison to both the preeclampsia and control groups and this finding was statistically meaningful (p<0.001). No meaningful differences were noted for the ALT levels of the preeclamptic patients upon comparison to those of eclamptic patients and the control group. However, ALT was found to be higher in eclamptic patients



Graph 2. Graphical portrayal of MMP and TIMP ratios in the study groups (see Graph 1 for abbreviations)

compared to control subjects and this difference was statistically meaningful (p=0.012). Finally, for LDH levels, no statistically meaningful difference was observed between the preeclamptic and control participants, but LDH was elevated in the eclampsia group compared to both the preeclampsia and control groups and this finding was significant (p<0.001).

In terms of MMP-2, meaningful differences were not noted among the study groups (p=0.786). While no meaningful differences in MMP-9 and TIMP-1 values existed between the preeclamptic and eclamptic groups, significantly lower MMP-9 and TIMP-1 levels were detected in these two groups upon comparison to the control group (p=0.001 for MMP-9, p<0.001 for TIMP-1). When preeclamptic patients were compared to the eclampsia and control groups, differences in TIMP-2 were not observed. However, in the eclamptic group, TIMP-2 values were seen to be significantly lower compared to the values observed for control subjects (p=0.04) (table 1 and graph 1).

For the ratio of MMP-2 to TIMP-2, meaningful differences were not noted among the three considered groups (p=0.788). When the groups were compared in terms of the MMP-9/TIMP-1 ratio, a significant difference was not observed between the preeclampsia and control groups. However, this ratio was elevated in the eclampsia group upon comparison to the preeclampsia and control groups and this finding was significant (p=0.011) (table 2 and graph 2).

Discussion

This research was designed with the aim of identifying interactions between MMP-2 and MMP-9, which have been found to participate in a wide array of physiological and pathological

Table 2. Comparison of MMP and TIMP Ratios

		n	Average	Standard deviation	Minimum	Maximum	p
MMP-2/TIM-2	Eclampsia	28	0.0801	0.15272	0.01	0.85	0.788
	Preeclampsia	28	0.0623	0.04143	0.01	0.20	
	Control	24	0.0750	0.05663	0.02	0.23	
	All patients	80	0.0723	0.09773	0.01	0.85	
MMP-9/TIM-1	Eclampsia	27	3.2801	2.98062	0.99	14.97	0.011
	Preeclampsia	28	1.9128	1.11468	0.00	6.11	
	Control	23	1.6306	1.52485	0.30	7.53	
	All patients	78	2.3029	2.15059	0.00	14.97	

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	All patients	78	2.3029	2.15059	0.00	14.97	

events in the pathogenesis of eclampsia and preeclampsia, together with their respective tissue inhibitors, TIMP-2 and TIMP-1.

The biological effects of MMPs are believed to be primarily due to the structural cumulative reorganization of vessel walls. Since physiological vascular reorganization is an important adaptive mechanism during pregnancy, this mechanism may be compromised in women with preeclampsia (10).

Palei et al., using the gelatin zymography technique, found higher levels of pro-MMP-9 and pro-MMP-9/TIMP-1 in cases of gestational hypertension compared to normal pregnancies. They also compared preeclamptic and normotensive pregnant women and subsequently reported no meaningful difference in circulating pro-MMP-9 (10). In the research presented here, on the contrary, MMP-9 and TIMP-1 were found to be significantly decreased in the preeclampsia group upon comparison to healthy pregnant women (p=0.001). However, for MMP-9/TIMP-1 ratios, values that provide information about net MMP-9 activities, consistently with the findings reported by Palei et al., we obtained no statistical difference between the preeclampsia and control groups. Additionally, in the present work, while no statistical difference in MMP-9 was noted for the eclampsia group upon comparison to the preeclampsia group, MMP-9 levels were seen to be significantly lower for these groups upon comparison to the control group (p=0.001).

Palei et al. stated that it was not possible to show that there was no difference between normotensive pregnancies, gestational hypertensive pregnancies, and preeclamptic pregnancies in terms of TIMP-2 levels and pro-MMP-2/TIMP-2 ratios reflecting net MMP-2 activity, or pro-MMP-2 concentrations (10). In our study, no difference was found in MMP-2, TIMP-2, or MMP-2/TIMP-2 ratios between preeclamptic and healthy participants.

Narumiya et al. showed that values of plasma MMP-2 expression were elevated among preeclamptic patients (15). However, some features of their study, such as the small sample sizes (preeclampsia patients = 12, normal pregnancies = 12) and the fact that plasma

samples were taken close to the time of delivery or expected delivery, with the possibility of plasma MMP-2 levels being affected during labor, make it difficult to compare their results to ours (15).

Myers et al. studied MMP-2, TIMP-1, and MMP-2/TIMP-1 in plasma samples that were collected from pregnant women who had developed preeclampsia at 22 gestational weeks, at 26 gestational weeks, and at the time of preeclampsia diagnosis or delivery (average of 36 weeks) (16). MMP-2 was seen to be more highly elevated at 22 weeks among patients who subsequently developed preeclampsia, followed by an additional increase at the time of diagnosis. However, such increases were not observed at week 26. The MMP-2/TIMP-1 ratio was noted to be high during all three periods considered in that study. These results reported by Myers et al. may have been due to the wide gestational age range in their study, as it has been demonstrated that MMP-2 levels change with gestational age, while our results may be due to the inclusion of only patients beyond 35 weeks of gestation in our study (16).

In the present work, statistically meaningful differences were not observed among the groups (eclampsia = 28, preeclampsia = 28, normal pregnancy = 24) in terms of MMP-2 or MMP-2/TIMP-2, with the latter reflecting net MMP-2 activity ($p=0.786$). No meaningful differences were noted for the eclampsia and control groups when both of them were compared to preeclamptic patients in terms of TIMP-2, but significantly lower TIMP-2 values were found in the eclamptic group than in the control group ($p=0.04$).

While no statistically meaningful differences were noted between the preeclamptic and eclamptic groups for MMP-9 or TIMP-1, significantly lower MMP-9 and TIMP-1 levels were detected in these two groups upon comparison to the control subjects ($p=0.001$ for MMP-9, $p=0.000$ for TIMP-1). In addition, while the MMP-9/TIMP-1 ratio was seen to be elevated with statistical significance in the eclampsia group upon comparison to the preeclampsia and control groups ($p=0.011$), such a difference was not detected between the preeclampsia and control group participants.

The interactions between MMPs and TIMPs in the pathogenetic processes of gestational hypertension and preeclampsia or eclampsia will continue to be a subject of interest in relation to many other popular medical topics (e.g., cardiovascular diseases, oncology, and embryogenesis). We compared the results of our study, in which we evaluated the plasma MMP-2, MMP-9, TIMP-1, and TIMP-2 values of women with preeclampsia, eclampsia, and healthy pregnancies in the same gestational week with previous findings in the literature, and, as described above, we found that our results were compatible with some studies and inconsistent with some others.

Imbalances between MMP-9 and TIMP-1 could be used for predicting preeclampsia and especially eclampsia. However, we believe that there is still a need for larger clinical studies describing these interactions in more depth.

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