Original Article

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Assessment of Matrix Metalloproteinase-1 and its Tissue Inhibitor of Metalloproteinase-1 in Pre-eclampsia

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Abstract

Introduction: Pre-eclampsia is a systemic inflammatory condition associated with maternal endothelial dysfunction. Matrix metalloproteinase (MMP) results in a poor trophoblastic invasion of the maternal spiral arteries, poor fetoplacental perfusion, and release of factors which affect the vascular tone and remodeling.

Objectives: To compare the serum values of MMP-1, tissue inhibitor of metalloproteinase (TIMP)-1, and their ratio in the second and third trimester of normal and pre-eclamptic pregnancy.

Materials and Methods: 30 females progressing to normal pregnancy were compared with 16 females who developed pre-eclampsia. MMP-1 and TIMP-1 concentrations were measured in serum samples (II and III trimester) of the females by enzyme linked immuno-sorbent assay.

Results: There was no significant difference in the levels of MMP-1, TIMP-1, and ratio of MMP-1 and TIMP-1 in pre-eclamptic and normal pregnancy females.

Conclusion: There is a lack of alteration in the levels of MMP-1, TIMP-1, and their ratio during the progression of pre-eclampsia when compared with normal pregnancy. Further studies with a larger sample size are required to validate this data.

Key words: Pre-eclampsia, Matrix metalloproteinase-1, Tissue inhibitor of metalloproteinase-1

INTRODUCTION

Pre-eclampsia is a systemic inflammatory condition characterized by high blood pressure and excess protein in the urine and is a leading cause of maternal and neonatal problems. The mechanisms that contribute to the disturbed endothelial homeostasis in the patho-physiology of pre-eclampsia remain unclear.[1] The main pathogenic feature of pre-eclampsia is maternal endothelial dysfunction that results from impaired angiogenesis and reduced endothelial repair capacity. It is proposed that the trophoblastic invasion into the spiral arteries insufficiently impacts the process of vascular remodeling resulting in the hypertensive disease of pregnancy.

Reactive oxygen species and tumor necrosis factor-α have been implicated in the pathogenesis of pre-eclampsia.[2] They tend to induce vascular expression of extracellular matrix proteins, particularly matrix metalloproteinases (MMPs).[3] Beyond their matrix remodeling properties, MMPs are involved in short-term biological processes, including regulation of vascular reactivity and leukocyte activation.[4] Recently, the role of MMPs in the pathogenesis of pre-eclampsia has aroused interest. MMPs are zinc- and calcium-dependent enzymes playing an important role in physiological as well as pathological mechanisms.[5] MMPs are implicated in the pathogenesis of angiogenesis and vascular remodeling by degrading extracellular matrix proteins associated with pre-eclampsia.[6] Evidence also suggest that decreased activity of MMPs results in poor trophoblastic invasion of the maternal spiral arteries, poor fetoplacental perfusion, and release of certain factors

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which affect the vascular tone and remodeling.[7] Specific endogenous inhibitors that bind MMPs are tissue inhibitors of metalloproteinases (TIMPs), and their expression is regulated during the development and tissue remodeling. The zymolytes of MMP-1 are collagen and metagelatin, which play an important role in the trophoblastic invasion[8] and are important factors in the regulation of trophoblastic invasion. MMPs and TIMPs together form a balance to maintain normal pregnancy and placental development.

Studies have indicated variations[9,10] in the levels of MMP-1 and TIMP-1 in the maternal umbilical cord serum, trophoblasts, and decidua in pre-eclampsia. Little is known regarding the serum levels of MMP-1 and TIMP-1 with the progression of pregnancy leading to pre-eclampsia, and it remains uncertain whether enhanced levels are present before the clinical signs of pre-eclampsia develop. With this in mind, the present study was carried out to assess the serum levels of MMP-1 and TIMP-1 trimester-wise in normal pregnancy and pre-eclampsia.

MATERIALS AND METHODS

Study Population

A prospective study approved by the Institutions Ethics Committee (NKPSIMS/7/2010 dated 20/8/2010) was carried out at NKP salve Institute of Medical Sciences, Nagpur, India. 30 primigravidas with uncomplicated normal pregnancies in the first trimester who were followed until the last trimester (control) and 16 primigravidas who developed pre-eclampsia (study group) in the third trimester were selected by random sampling.

Inclusion Criteria

All subjects within the age group of 18-35 years were selected for the study. Pre-eclampsia was defined as persisting elevated diastolic blood pressure (90 mmHg), proteinuria (>300 mg in a 24 urine sample), and the presence of edema. Subjects willing to participate were included in the study. The females with normal pregnancy were included as the control group.

Exclusion Criteria

Subjects with non-confirmed pre-eclampsia, essential hypertension, malaria, hemolytic anemia, and any other infections such as urinary tract infection or upper respiratory tract infection.

Collection of Sample

2 ml blood sample was collected from the antecubital vein under strict aseptic precautions (between 24-26 weeks and 36-38 weeks of pregnancy). The blood was allowed to clot for 30 min at room temperature and centrifuged at 3000 rpm for 15 min. The serum was then pipetted and placed in sterilized vials free of endotoxins at −20°C until analysis.

Biochemical Analysis

Human MMP-1 and TIMP-1 enzyme linked immunosorbent assay (ELISA) was measured by Ray Bio (Ray Biotech, Inc., USA). This kit is an in vitro ELISA that quantitatively measures human MMP-1 and TIMP-1 in serum, plasma, cell culture supernatants, and urine. This assay uses human-specific MMP-1 and TIIMP-1 antibodies coated on a 96-well plate. Standards and samples were pipetted into the wells, and the MMP-1 and TIMP-1 bound to the wells through the immobilized antibody. The wells were washed, and biotinylated antihuman MMP-1 and TIMP-1 antibodies were added. After washing to remove unbound biotinylated antibody, we pipette HPR-conjugated streptavidin into the wells. The wells were washed, and subsequently, a tetra methylbenzidine substrate solution was added to the wells. Color developed in proportion to the amount of MMP-1 and TIMP-1 bound to the well. The stop solution changed the color from blue to yellow, and the color intensity was measured at 450 nm. MMP-1 and TIMP-1 concentration were determined using a standard curve.

Statistical Analysis

Statistical analysis was performed using EPI info software. *P* value was calculated. A value of $P < 0.001$ was considered to be significant.

RESULTS

As depicted in Table 1 and Figure 1, we observed that there was no statistically significant difference in the levels of MMP-1, TIMP-1, and MMP-1/TIMP-1 in the second and third trimester of females with normal pregnancy or those who developed pre-eclampsia.

DISCUSSION

Pre-eclampsia, characterized by hypertension, proteinuria accounts for considerable mortality and morbidity. In a normal pregnancy, the luminal diameter of the spiral

Table 1: Comparison of MMP-1 and TIMP-1 levels among the study groups

Groups (ng/ml)	Normal pregnancy		Pre-eclampsia	
	II trimester	III trimester	II trimester	III trimester
MMP-1	4.26±2.48	4.99±2.75	4.38±1.15	4.83±2.84
TIMP-1	34.98±8.66	32.97±7.08	36.69±9.007	36.69±8.97

MMP-1: Matrix metalloproteinase-1, TIMP-1: Tissue inhibitor of metalloproteinase-1

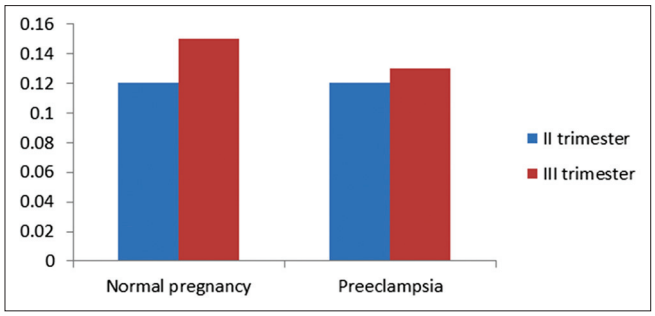

Figure 1: Comparison of matrix metalloproteinase-1/tissue inhibitor of metalloproteinase-1 ratio among the study groups

arteries is increased, and the vascular smooth muscle is replaced by trophoblastic cells. This process is deficient in pre-eclampsia. Though numerous pathophysiologies have been proposed for the development of pre-eclampsia, a poorly perfused fetoplacental unit is considered to be the major cause. One of the emerging pathway involved is the MMPs. MMPs are known to play an important role in endothelial invasion, angiogenesis, and in tumor progression.[11] MMP-1 or interstitial collagenase is a proteolytic enzyme produced in tissues under conditions of inflammation which break down collagen. TIMPs are known to regulate this proteolytic activity of MMPs.

Our study demonstrates that there is no significant difference in the serum values of MMP-1, TIMP-1, or there ratio in pre-eclamptic females when compared with that of normal pregnancy. Moreover, according to our findings, there is no statistical difference in the MMP-1 and TIMP-1 values in females as the pregnancy proceeds from second to the third trimester. This may be attributed to the small sample size of the study.

Galewska *et al.*[12] have suggested a decrease in the content of MMPs in the umbilical cord artery in pre-eclamptic females. Deng *et al.*[9] and Jurajda *et al.*[13] have demonstrated that the expression levels of MMP-1 in the umbilical cord blood, placenta, and deciduas of patients with hypertension disorders in pregnancy were significantly lower than compared with normal pregnancy. The invasion ability of the trophoblasts was lower in hypertensive disorders and TIMPs bind to MMPs in 1:1 stoichiometry, and hence decrease in the levels of MMP-1 and TIMPs.

The Virginia Commonwealth University School of Medicine Researchers recently has suggested that there is a significant increase in the levels of MMP-1 in the blood vessels of women with pre-eclampsia. Pre-eclampsia is said to be associated with an imbalance in collagen-regulating genes that favor collagen breakdown. Leakage of protein out of the blood vessels into the surrounding tissue (edema) and through the blood vessels of the kidney and into the urine (proteinuria) may be due to the increase in the MMP-1 levels, which compromises the integrity of the mother's blood vessels. MMP-1 also causes blood vessel contraction by the activation of a receptor known as protease-activated receptor-1 which is also found in high concentrations in pre-eclampsia.

Merchant and Davidge[14] have suggested that MMP enhanced the myogenic tone and endothelium-dependent relaxation in vessels suggesting a greater role of MMPs in mediating vasodilation. This may be due to the depolarization of the vascular smooth muscle cell and an increase in intracellular calcium.[15]

CONCLUSION

Variation in research data is available regarding the role of MMPs and TIMPs in pre-eclampsia. Our study is not able to demonstrate the effectiveness of the measurement of these blood parameters specially MMP-1 and TIMP-1 in pre-eclampsia. Further studies with a larger sample size may be taken into consideration to validate the results and to assess the usefulness of MMPs as a predictive marker of pre-eclampsia.

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