Evaluation of Metalloproteinases 2 and 9 and Their Inhibitors in Physiologic and Pre-eclamptic Pregnancy

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Matrix metalloproteinases (MMPs) are a family of zinc and calcium-dependent endopeptidases involved in remodeling and physiological homeostasis of extracellular matrix (ECM). The metalloproteinases activity is predominantly modulated by specific tissue inhibitors of matrix metalloproteinases (TIMPs). The balance between MMPs and TIMPs is likely to play an important role in remodeling uterine arteries in pregnancy, and it may represent means by which vasodilatation is maintained in later pregnancy. Moreover, increased levels of MMPs and in particular MMP-2 play a role in the vascular alterations induced by hypertension. The aim of this study was the evaluation of MMP-2 and -9, along with their inhibitors TIMP-1 and -2, in pre-eclamptic women compared with normotensive pregnancy and non-pregnant women. Fourteen pre-eclamptic women were compared with 37 normotensive women in different gestational age and 21 non-pregnant women. Multiplexed sandwich enzyme-linked immunosorbent assay was used to measure

MMPs and TIMPs simultaneously. MMP-2 levels were significantly higher in pre-eclamptic women vs. both non-pregnant and physiologic pregnant women. MMP-9 concentrations were significantly higher in physiologic pregnant vs. non-pregnant women. The serum levels of TIMP-1 were significantly higher in pre-eclamptic vs. both non-pregnant and physiologic pregnant women. TIMP-2 values were higher in physiologic pregnant women and pre-eclamptic women vs. non-pregnant women. A positive correlation between MMP-9 values and gestational age was observed in normal pregnant women. Results of the present investigation confirm that MMP-2 and TIMP-1 values are significantly higher in preeclampsia. We confirm that the modification of the fine balance between MMPs and their inhibitors plays a greater role in the structural and functional vascular changes of women with complicated pregnancies. *J. Clin. Lab. Anal.* 23:88–92, 2009. © 2009 Wiley-Liss, Inc.

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INTRODUCTION

Matrix metalloproteinases (MMPs), a family of zinc and calcium-dependent endopeptidases involved in remodeling and physiological homeostasis of extracellular matrix (ECM) (1), are considered reliable indices of vascular tissue matrix turnover (2). Moreover, they are implicated in several pathologic processes characterized by either the degradation of connective tissue matrix or a proinflammatory state (3). MMPs are grouped into several subtypes, including collagenases, gelatinases, stromelysins, and other enzymes (4). Targets of these degradative enzymes include structural ECM molecules

(i.e., interstitial collagens, laminins, fibronectins, and heparan sulfate proteoglycans), cell–cell adhesion molecules, cell surface receptors, growth factors, cytokines, and chemoattractant proteins, as well as other proteinases (1). The metalloproteinases activity is

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predominantly modulated by specific inhibitors called tissue inhibitors of matrix metalloproteinases (TIMPs) (5). Four subgroups of specific TIMPs have been described so far (6), whose physiological activity is mediated through a covalent binding to the corresponding enzyme.

Different studies on animal models have highlighted a tightly regulated balance between the activated MMPs involved in degradation of connective tissue matrices and their relative inhibitors. In particular, the proliferation and differentiation of uterine stromal cells (decidualization) are regulated in part by the expression of matrix MMPs and TIMPs (7). Moreover, it has been previously proposed that MMPs could play a greater role in the vasculature of women with preeclampsia compared with non-pregnant and normal pregnant women, by enhancing myogenic tone and impairing endothelium-dependent relaxation (8). Regardless of the available evidence, there is still debate, however, on the levels of MMPs in different gestational age. The aim of this study was hence the evaluation of MMP-2 and -9 and TIMP-1 and -2 in pre-eclamptic women compared with normotensive pregnancy in different gestational age and non-pregnant women.

MATERIALS AND METHODS

Fourteen pre-eclamptic women (proteinuria ≥ 300 mg/24 hr, two readings of systolic blood pressure ≥ 140 mmHg, and diastolic blood pressure ≥ 90 mmHg) (9) were compared with 37 normotensive pregnant women in different gestational age (10 in first, 10 in second, and 17 in third trimester of pregnancy), and 21 non-pregnant women recruited among healthy hospital personnel. No normotensive or non-pregnant women were currently taking medications. Eight out of 14 pre-eclamptic women were treated with antihypertensive drugs. Renal and liver function was normal in all 14 pre-eclamptic patients, as established by routine laboratory tests. Each patient and control gave an explicit and written informed consent for participating in the study,

which has been approved by our departmental ethical committee. Blood pressure was recorded using an automatic oscillometric device (DYNAMAP) with the subjects in the supine position for at least 15 min.

Blood samples were collected in the morning after an overnight fast and a resting period of 20 min in vacuum tubes containing no additives (Becton-Dickinson, Oxford, UK). After centrifugation at $1500 \times g$ for 10 min at room temperature, serum was separated, stored in aliquots, and kept frozen at -70°C until measurement. SearchLight Human MMP Array 1 (Pierce, Rockford, IL) was used to measure MMPs and TIMPs simultaneously. The assay consists of multiplexed sandwich enzyme-linked immunosorbent assay for the quantitative measurement of four proteins in each sample: gelatinases (MMP-2 and MMP-9) and endogen inhibitors (TIMP-1 and TIMP-2). Each sample was assayed twice and the mean value of both measurements was used. The enzyme-substrate reaction produces a chemiluminescent signal detected with a cooled CCD camera (Pierce). The images were analyzed by ArrayVision version 8.0 software (Imaging Research). The mean intra-assay coefficient of variation (CV) was $<10\%$ for all biomarkers. Serum creatinine, glucose, and albumin were measured on the Modular System P (Roche Diagnostics GmbH), employing proprietary reagents. Total imprecision, as expressed by the relative CV, was always $<2\%$ for the three analytes tested. Values were finally reported as median (interquartile range). Results of measurements and relative values' distributions between groups were compared by Kruskal Wallis and Mann-Whitney *U* test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Main demographic and biochemical features of the study populations are given in Table 1. Results of MMP and TIMP measurements are shown in Figure 1 and Table 2.

TABLE 1. Demographic and Biochemical Variables in Non-pregnant, Normal Pregnant, and Preeclamptic women

	Non-pregnant women	Normal pregnant women	Preeclamptic women	<i>P</i> -value K-W
<i>n</i>	21	37	14	—
Age (y)	30 (28–36)	30 (27–34)	32 (29–38)	0.473
SBP (mmHg)	120 (110–130)	120 (110–130)	150 (140–160) ^{†‡}	0.001
DBP (mmHg)	80 (70–80)	80 (70–80)	90 (90–100) ^{†‡}	0.001
Creatinine ($\mu\text{mol/L}$)	62.7 (57.3–70.0)	50.9 (44.5–57.3) [†]	56.4 (48.4–62.5)	0.001
Glucose (mmol/L)	4.6 (4.3–4.9)	4.4 (4.0–4.7)	5.5 (4.6–7.1) ^{†‡}	0.003
Albumin (g/L)	43.7 (42.3–45.4)	36.9 (35.8–41.1) [†]	30.9 (28.8–31.8) ^{†‡}	0.001

Data are expressed as median (interquartile range) or number (percent). SBP indicated systolic blood pressure and DBP, diastolic blood pressure. *P*-values are evaluated by Kruskal-Wallis test. *P*-values between groups are evaluated by Mann-Whitney *U* test. [†] indicated $P < 0.05$ vs. non-pregnant women and [‡] indicated $P < 0.05$ vs. physiologic pregnancy.

Fig. 1. Results of MMP and TIMP measurements non-pregnant ($n = 21$), normal pregnant ($n = 37$), and pre-eclamptic women ($n = 14$). P -values are evaluated by Kruskal Wallis test. P -values between groups are evaluated by Mann–Whitney U test.

TABLE 2. Results of MMP and TIMP Measurements in Non-pregnant, Normal Pregnant, and Preeclamptic women

	Non-pregnant women	Normal pregnant women	Preeclamptic women	P -value K-W
MMP-2 (ng/mL)	568 (454–720)	669 (560–760)	834 (656–1002) ^{†‡}	0.003
MMP-9 (ng/mL)	215 (129–339)	390 (277–569) [†]	290 (280–470)	0.007
TIMP-1 (ng/mL)	180 (155–195)	148 (121–188)	213 (212–220) ^{†‡}	0.006
TIMP-2 (ng/mL)	200 (169–224)	228 (207–267) [†]	232 (225–245) [†]	0.040

Data are expressed as median (interquartile range). P -values are evaluated by Kruskal Wallis test. P -values between groups are evaluated by Mann–Whitney U test. [†] indicated $P < 0.05$ vs. non-pregnant women and [‡] indicated $P < 0.05$ vs. normal pregnant women.

The serum concentrations of MMP-2 were significantly higher in pre-eclamptic women vs. both non-pregnant (834 vs. 568 ng/mL, $P = 0.001$) and physiologic pregnant women (834 vs. 669 ng/mL, $P = 0.01$). MMP-2 values in the first trimester of pregnancy were significantly higher than those observed in non-pregnant women (698 vs. 568 ng/mL, $P = 0.03$). Serum concentrations of MMP-9 were significantly higher in physiologic pregnant vs. non-pregnant women (390 vs. 215 ng/mL, $P = 0.003$). The serum concentrations of TIMP-1 were significantly higher in pre-eclamptic vs. both non-pregnant (213 vs. 180 ng/mL, $P = 0.02$) and physiologic pregnant women (213 vs. 148 ng/mL, $P = 0.007$). TIMP-2 values were higher in physiologic pregnant women vs. non-pregnant women (228 vs. 200 ng/mL, $P = 0.03$) and in pre-eclamptic women vs. non-pregnant women (232 vs. 200 ng/mL, $P = 0.02$). A positive correlation between MMP-9 values and gestational age was observed in

normal pregnant women ($r = 0.36$, $P = 0.02$). No correlations were observed between gestational age and MMP-2, TIMP-1, or TIMP-2.

DISCUSSION

The remodeling of uterine spiral arteries during normal implantation is essential for successful pregnancy (10). The balance between MMPs and TIMPs is likely to play an important role in remodeling uterine arteries in pregnancy, and it may represent means by which vasodilatation is maintained in later pregnancy (11). MMP-2 and MMP-9 efficiently degrade type IV collagen, a major component of the basement membrane, and both are associated with active neovascularization (12). MMPs and their inhibitors, along with the vascular endothelial growth factor (VEGF) system, play also an important role throughout various stages of

pregnancy, including embryo implantation, trophoblastic invasion, placentation in early gestation, cervical dilatation in later gestation, and feto-maternal membrane lysis (13,14).

It has been previously reported that increased net MMP-2 activity may contribute to the endothelial dysfunction that is central to the pathophysiology of preeclampsia (15). Accordingly, we have observed increased levels of MMP-2 in pathologic pregnancies with respect to normal pregnant women. Narumiya et al. have suggested that VEGF could promote secretion of MMP-2 from endothelial cells that, in turn, might alter vascular function through cleavage of vasoactive peptides, resulting in increased vasoconstriction and reduced vasodilation in women with preeclampsia (16), as already observed in other pathologies (17). However, in accordance with Maymon et al. (18), we failed to observe a significant correlation between MMP-2 concentration and the gestational age, which is basically in agreement with the key role of this metalloproteinase. In normal pregnancy, the levels of MMP-2 are finely regulated during all gestational time, but they are significantly higher in women with preeclampsia, demonstrating a substantial impairment of this balance (16).

On the contrary, we observed significantly increased MMP-9 in physiologic pregnancy, as well as a significant positive correlation between MMP-9 and the gestational age ($r = 0.36$, $P = 0.02$), supporting a major involvement of MMP-9 in the physiologic pregnancy. Conversely, we recorded lower levels of MMP-9 in complicated pregnancies as compared with the physiological ones. This is in agreement with the previous observation that women with preeclampsia display reduced MMP-9 concentrations in placenta extract when compared with normal pregnant women (19).

We have also observed increased TIMP-2 during physiologic placentation, providing further support to the hypothesis that this inhibitor plays a pivotal role in decidualization. Hurst and Palmay observed that TIMP-2 is strongly localized in the primary decidual tissue and modulates the trophoblastic invasion (20), whereas Okamoto et al. demonstrated that both the mRNA and protein concentrations of TIMP-2 are markedly decreased in hydatidiform mole and choriocarcinoma tissues, suggesting that the imbalance of MMP-TIMP production may be involved in the pathogenesis of these diseases (21). Accordingly, as preeclampsia is a possible consequence of suboptimal trophoblast invasion, MMP-2 and TIMP-1 are significantly elevated in the plasma of pre-eclamptic women and are mediators of vascular reactivity (16). An imbalance in the MMP-2:TIMP-1 ratio was also observed in patients who develop preeclampsia (15), and pre-eclamptic endothelial cells

significantly demonstrate enhanced MMP-2 and TIMP-1 release as compared with normal cell (22). Therefore, the net increases in the metalloproteinases activity over their inhibitors may contribute to the endothelial dysfunction that is central to the pathophysiology of preeclampsia. This is consistent with the evidence that untreated essential hypertension is characterized by an increase in collagen synthesis, degradation, and inhibition of degradation, highlighting that TIMP-1 acts as a mediator of fibrosis by inhibiting MMPs activity and leading to fibrosis and left ventricular diastolic dysfunction (23).

Reproductive tissues are unique structures that exhibit substantial remodeling during menstrual cycles and pregnancy. The fine balance between MMPs and their inhibitors plays a greater role in the vasculature remodeling of women with normal and complicated pregnancies. Results of the present investigation confirm that MMP-2 and TIMP-1 values are significantly higher in preeclampsia. For that reason, the evaluation of the balance between MMPs and their inhibitors may be a valuable aid to assist the clinician in monitoring the physiologic and pathological evolution of placentation.

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