Comparative assessment of matrix metalloproteinase (MMP)-2 and MMP-9, and their inhibitors, tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2 in preeclampsia and gestational hypertension

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Abstract

Objectives: To compare the circulating levels of matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitors of metalloproteinase (TIMP)-1, TIMP-2, and the MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios in preeclampsia and gestational hypertension with those found in normotensive pregnancies.

Design and methods: We studied 83 pregnant women (30 healthy pregnant women with uncomplicated pregnancies, 26 with gestational hypertension, and 27 with preeclampsia) and 30 healthy nonpregnant women in a cross-sectional study. MMP and TIMP concentrations were measured in plasma samples by gelatin zymography and ELISA, respectively.

Results: We found higher plasma pro-MMP-9 levels, and higher pro-MMP-9/TIMP-1 ratios in women with gestational hypertension (95%-CI: 1.031 to 2.357, and 0.012 to 0.031, respectively), but not with preeclampsia, compared with those found in normotensive pregnant women (95%-CI: 0.810 to 1.350, and 0.006 to 0.013, respectively; both \( P < 0.05 \)). We found no significant differences in pro-MMP-2 levels (\( P > 0.05 \)).

Conclusions: The higher net MMP-9 (but not MMP-2) activity in gestational hypertension compared with normotensive pregnancy suggests that MMP-9 plays a role in the pathophysiology of gestational hypertension. Conversely, the lack of such alterations in preeclampsia is consistent with the notion that different pathophysiological mechanisms are involved in these hypertensive disorders.

Keywords: Gestational hypertension; Metalloproteinases; Preeclampsia; TIMPs

Introduction

Hypertensive disorders are common complications affecting 5% to 10% of pregnancies [1] and a major cause of preterm delivery [2]. While various hypotheses have been explored to explain pregnancy-induced hypertension (PIH; which includes preeclampsia and gestational hypertension) and chronic hypertension, the pathophysiology of these conditions remains to be determined [3]. In this regard, there is growing evidence suggesting that decreased activity of matrix metalloproteinases (MMPs) could result in poor trophoblast invasion of maternal spiral arteries, thus leading to poor fetoplacental unit perfusion and release of placental factors that affect the vascular tone and remodeling [4]. In addition, it has recently been suggested that MMPs (especially MMP-2) play a greater role in mediating vasodilation in preeclamptic pregnancies compared with normotensive pregnancies [5]. Together, these findings are consistent with the notion that MMPs play significant roles in both acute and chronic regulation of the cardiovascular system [6].

Giving support to these previous reports, a few clinical studies described changes in the circulating levels of MMPs (especially MMP-2 and MMP-9) and their endogenous inhibitors (tissue inhibitors of metalloproteinases; TIMPs) in women with PIH [7–10]. This is important because measuring the plasma levels...
of MMPs and TIMPs may help to elucidate important mechanisms possibly involved in the pathogenesis of PIH. Since TIMP-1 and TIMP-2 are major inhibitors of MMP-9 and MMP-2, respectively [11], the assessment of MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios may lead to improved information regarding the net activity of these two MMPs. In this regard, there is only one study showing lower MMP-9/TIMP-1 ratios in the plasma from women with gestational hypertension compared with those found in the plasma of normotensive pregnant women [7]. Unfortunately, MMP-2/TIMP-2 ratios were not examined in this study [7]. Therefore, additional information is necessary to evaluate the possible contribution of MMP-2 and MMP-9 to gestational hypertension. With respect to preeclampsia, only two studies showed similar circulating MMP-9 and TIMP-1 concentrations in preeclamptic and in normotensive pregnant women at 37–38 weeks gestation, although MMP-9/TIMP-1 ratios were not examined in these studies [8,10]. In addition, although two studies by the same group [8,9] showed increased MMP-2 levels in the plasma from preeclamptic women, no previous study has compared the circulating levels of TIMP-2 levels or MMP-2/TIMP-2 ratios in preeclamptic women with those found in normotensive pregnancies.

In the present study, we hypothesized that altered MMP-2/TIMP-2 and possibly MMP-9/TIMP-1 ratios would be found in PIH (both gestational hypertension and preeclampsia) compared with normotensive pregnancy. Therefore, the aim of our study was to compare the circulating levels of MMP-2, MMP-9, TIMP-1, TIMP-2, and the MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios in preeclampsia and gestational hypertension with those found in normotensive pregnancies. We have also measured these levels in a group of healthy nonpregnant women.

Methods

Subjects

Approval for use of human subjects was obtained from the Institutional Review Board at the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil. This is a cross-sectional study where there was one sampling around 32 weeks at the exception of non pregnant normotensive women. All patients were enrolled in the Department of Obstetrics and Gynecology, University Hospital of the Faculty of Medicine of Ribeirao Preto from October/2006 to February/2007. We studied 83 pregnant women (30 healthy pregnant women with uncomplicated pregnancies, 26 with gestational hypertension, and 27 with preeclampsia) and 30 healthy non pregnant women randomly selected from the local population and unrelated to the patients. Hypertensive disorders were defined in accordance with the guidelines of the NHBPEP (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy) [12]. Gestational hypertension was defined as pregnancy-induced hypertension (≥ 140 mmHg systolic or ≥ 90 mmHg diastolic on 2 or more measurements at least 6 h apart) without significant proteinuria in a woman after 20 weeks of gestation, and returning to normal by 12 weeks post-partum. Preeclampsia was defined as increased blood pressure with significant proteinuria (≥ 0.3 g/24 h) in a woman after 20 weeks of gestation. No women with pre-existing hypertension, with or without superimposed preeclampsia, were included in the present study. Exclusion criteria included twin or multiple pregnancies or any evidence of previous medical illness.

While the patients were followed as outpatients (at about 32 weeks of gestational age) maternal venous blood samples were collected into standard Vacutainer tubes (Becton-Dickinson, Brazil) containing sodium/potassium EDTA, and antihypertensive treatment with methydopa was begun whenever indicated. The main reason for sampling pregnant women at about 32 weeks was that hypertensive (both preeclamptic and gestational hypertensive) women are at high risk of preterm delivery, and we wanted to examine MMPs/TIMPs in plasma samples from women sampled at comparable gestational ages. The tubes were centrifuged immediately at room temperature and plasma samples were stored for about 3–4 months at −70 °C until used to measure plasma MMP-2, MMP-9, TIMP-1, and TIMP-2 concentrations.

SDS-polyacrilamide gel electrophoresis (PAGE) gelatin zymography of MMP-9 and MMP-2

Gelatin zymography of MMP-9 and MMP-2 from plasma was performed as previously described [13–16]. Briefly, plasma samples were subjected to electrophoresis on 7% SDS-PAGE co-polymerized with gelatin (1%) as the substrate. After electrophoresis was complete, the gel was incubated for 1 h at room temperature in a 2% Triton X-100 solution, and incubated at 37 °C for 16 h in Tris–HCl buffer, pH 7.4, containing 10 mmol/L CaCl2. The gels were stained with 0.05% Coomassie Brilliant Blue G-250, and then destained with 30% methanol and 10% acetic acid. Gelatinolytic activities were detected as unstained bands against the background of Coomassie blue-stained gelatin. Enzyme activity was assayed by densitometry using a Kodak Electrophoresis Documentation and Analysis System (EDAS) 290 (Kodak, Rochester, NY) [17]. The pro form of MMP-2 and MMP-9 were identified as bands at 72 and 92 kDa, respectively, by the relation of log Mr to the relative mobility of Sigma SDS-PAGE LMW marker proteins. A representative zymogram of plasma samples is shown in Fig. 1.

Enzyme immunoassays of TIMP-1 and TIMP-2

The plasma concentrations of TIMP-1 and TIMP-2 were measured with commercially available enzyme-linked immunosorbent assay kits [18] (Amersham Biosciences UK Limited, UK) according to the manufacturer’s instructions.

Statistical analysis

With basis on previous studies [7], we calculated sample size taking into consideration that differences in MMP-9 corresponding to 60% of S.D. would be meaningful. Therefore, for α<0.05 and β>0.20, 20 or more subjects would be required.

Data were reported as the mean±S.D. or range and quartiles. The between group comparisons were assessed by Kruskall–
Wallis test, followed by Dunn’s selected pair comparisons (StatView for Windows, Cary, NC, USA). A probability value $b 0.05$ was considered the minimum level of statistical significance.

**Results**

Table 1 summarizes the clinical and laboratorial characteristics of the 113 subjects enrolled in the present study. There were no statistically significant differences in age, gestational age, heart rate, hemoglobin concentration, hematocrit, creatinine, and %nulliparous between hypertensive groups and the control group (all $P>0.05$). However, women with gestational hypertension had higher BMI than the other groups ($P<0.05$; Table 1). Higher systolic and diastolic blood pressure were found in women with gestational hypertension or with preeclampsia compared with the other groups (both $P<0.05$; Table 1). Lower birth weights and gestational ages at delivery were found in the group of preeclamptic women compared with the other groups ($P<0.05$; Table 1).

Gelatin zymography of plasma samples showed all forms of MMPs usually found in human plasma, including the homodimer of the pro-MMP-9 form (225 kDa), the pro-MMP-9 complexed with neutrophil gelatinase-associated lipocalin (NGAL) form (130 kDa), the pro-MMP-9 form (92 kDa) and the pro-MMP-2 (72 kDa) form (Fig. 1). Gelatinolytic activity was completely inhibited by 5 mM EDTA or 1 mM 1, 10-phenantroline (data not shown), thus confirming that these bands correspond to MMP activity. In addition, we have also found some bands between 92 and 72 kDa which were not inhibited by phenantroline and correspond to non-MMP gelatinases present in human plasma [19].

Interestingly, we found higher plasma levels of pro-MMP-9 in the group of women with gestational hypertension compared with those found in normotensive pregnant women ($P<0.05$; Fig. 2). In addition, women with gestational hypertension had higher pro-MMP-9/TIMP-1 ratios compared with those found in the normotensive pregnant controls ($P<0.05$; Fig. 2). No significant differences were found in pro-MMP-2 levels in the present study ($P>N 0.05$; Fig. 3). However, nonpregnant women had lower TIMP-2 levels and higher pro-MMP-2/TIMP-2 ratios than those found in normotensive pregnant controls (both $P<0.05$; Fig. 3).

**Discussion**

The main findings reported here are that women with gestational hypertension have higher plasma levels of pro-MMP-9/TIMP-1 ratio when preeclamptic women were compared with normotensive pregnant women, although higher TIMP-1 levels were found in preeclamptic compared with normotensive pregnant women ($P<0.05$; Fig. 2).

We found no significant differences in pro-MMP-2 levels in the present study ($P>0.05$; Fig. 3). However, nonpregnant women had lower TIMP-2 levels and higher pro-MMP-2/TIMP-2 ratios than those found in normotensive pregnant controls (both $P<0.05$; Fig. 3).

**Table 1**

Demographic characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Normotensive pregnant</th>
<th>Gestational hypertension</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.8±3.5</td>
<td>25.7±3.8</td>
<td>28.1±4.0</td>
<td>27.9±4.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1±1.2</td>
<td>23.6±3.6</td>
<td>32.7±4.9</td>
<td>24.9±4.8</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>–</td>
<td>32.9±3.5</td>
<td>32.2±4.9</td>
<td>32.4±3.6</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113.9±5.7</td>
<td>107.3±6.8</td>
<td>132.6±10.3</td>
<td>143.9±10.6*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77.1±4.8</td>
<td>70.0±4.1</td>
<td>82.9±8.4</td>
<td>91.8±9.5*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>78.8±4.7</td>
<td>84.6±5.6</td>
<td>82.8±5.5</td>
<td>79.2±5.7</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.2±1.5</td>
<td>12.2±1.3</td>
<td>11.7±1.3</td>
<td>12.8±1.2</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>38.6±3.1</td>
<td>34.7±3.8</td>
<td>35.0±3.7</td>
<td>37.7±3.2</td>
</tr>
<tr>
<td>24-h-Pr (mg/24 h)</td>
<td>ND</td>
<td>ND</td>
<td>127.6±67.9</td>
<td>1063.0±849.5</td>
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<tr>
<td>Creatinine (μmol/L)</td>
<td>61.2±19.5</td>
<td>56.7±15.9</td>
<td>54.1±11.5</td>
<td>57.6±13.3</td>
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<td>Nulliparous (%)</td>
<td>35</td>
<td>40</td>
<td>31</td>
<td>41</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>–</td>
<td>3223±540</td>
<td>3161±450</td>
<td>2200±462*</td>
</tr>
<tr>
<td>GAD (weeks)</td>
<td>–</td>
<td>39.3±0.9</td>
<td>38.7±0.7</td>
<td>34.2±1.8*</td>
</tr>
</tbody>
</table>

BMI, body mass index; GA, gestational age; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; Hb, hemoglobin concentration; Hct, hematocrit; GAD, gestational age at delivery; 24-h-Pr; 24-h proteinuria; ND: not determined (however, with negative dipstick test).

Values are the mean±S.D.

* $P<0.05$ vs. normotensive pregnant group.
and higher pro-MMP-9/TIMP-1 ratios compared with those found in the normotensive pregnant controls. In addition, although no significant differences were found in pro-MMP-2 levels, we found that normotensive pregnancy is associated with higher circulating TIMP-2 levels compared with those found in non pregnant women.

In the present study, we used gelatin zymography to examine whether PIH affects the circulating levels of MMP-2 and MMP-9 because altered expression or activity of these enzymes has been reported to play a role in a variety of pathological conditions including gestational[7–10,20] and cardiovascular diseases including hypertension [17,21,22]. Indeed, the circulating level of MMP-9 has been suggested to be a clinically relevant blood-borne biochemical marker of diagnostic and prognostic value in cardiovascular diseases [21,22]. We have also measured the concentrations of TIMP-1 and TIMP-2 in order to evaluate the MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios, which may lead to improved information regarding the net MMP activities. Curiously, the increased pro-MMP-9 levels and pro-MMP-9/TIMP-1

Fig. 2. Plasma Pro-MMP-9 and TIMP-1 concentrations, and Pro-MMP-9/TIMP-1 ratio in nonpregnant (N=30), normotensive pregnant (N=30), gestational hypertensive (N=26), and preeclamptic women (N=27). The box and whisker 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 the lowest values. *P<0.05 vs. normotensive pregnant, by Kruskall–Wallis test, followed by Dunn’s selected pairs comparisons.

Fig. 3. Plasma Pro-MMP-2 and TIMP-2 concentrations, and Pro-MMP-2/TIMP-2 ratio in nonpregnant (N=30), normotensive pregnant (N=30), gestational hypertensive (N=26), and preeclamptic women (N=27). The box and whisker 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 the lowest values. *P<0.05 vs. normotensive pregnant, by Kruskall–Wallis test, followed by Dunn’s selected pairs comparisons.
ratios that we found in gestational hypertension are in contrast with previously reported results of a similar cross-sectional study [7]. Although we have no precise explanation for these conflicting results, differences in the methods (zymography vs. ELISA) used to measure the circulating MMP-9 levels may be involved. In addition, the circulating levels of MMP-9 correlated positively with gestational age [10], and it is possible that differences in gestational ages between studies may have affected the results. To our knowledge, there is no other study examining circulating MMPs in gestational hypertension. Importantly, the increased pro-MMP-9 levels and pro-MMP-9/TIMP-1 ratios in gestational hypertension reported here are consistent with previous studies showing higher MMP-9 levels in hypertensive patients compared with normotensive controls [17,23,24]. Further studies on gestational hypertension are needed to confirm these findings.

Consistent with two previous studies [8,10], we found no significant differences in circulating pro-MMP-9 levels when preeclamptic and normotensive pregnant women were compared. Our findings of similar MMP-9/TIMP-1 ratios in these two experimental groups give further support to the suggestion that net MMP-9 activity in plasma of preeclamptic women is similar to that found in normotensive pregnancies [8,10]. Taken together, these findings suggest that MMP-9 is not involved in the pathogenesis of preeclampsia.

The lack of significant differences in pro-MMP-2 levels between normotensive and hypertensive pregnancies (both gestational hypertension and preeclampsia) reported here are in contrast with two small previous studies by the same group [8,9]. Interestingly, these authors showed higher MMP-2 levels in preeclamptic women at 22 and at 36 week gestation, but not at 26 weeks [8], thus suggesting that gestational age has a major effect on MMP-2 levels. We have no obvious explanation for the differences between our results and those previously reported [8,9], it is possible that differences in gestational ages affect the results reported in these studies. However, in addition to the similar pro-MMP-2 concentrations, the comparable TIMP-2 levels and pro-MMP-2/TIMP-2 ratios in normotensive and hypertensive pregnancies reported here suggest that there are no differences in net MMP-2 activity among these groups.

It is well known that essential and chronic hypertensive women may become normotensive in pregnancy up to 32 weeks. However, although this is a cross-sectional study, the pregnant women enrolled in the present study were followed in our outpatient clinic from the beginning of their pregnancies. Therefore, it is not possible that other concurrent hypertensive conditions such as chronic hypertension may have affected the results reported here.

In conclusion, we found evidence indicating higher net MMP-9 (but not MMP-2) activity in gestational hypertension compared with that found in normotensive pregnancy. We also found lack of evidence for altered net MMP-9 or MMP-2 activities in preeclampsia. The lack of such alterations in preeclampsia is consistent with the notion that different pathophysiological mechanisms are involved in these hypertensive disorders. It is possible that higher net MMP-9 activities in gestational hypertensive pregnancies lead to accelerated cleaving of big endothelin-1 [25] to yield higher concentrations of medium endothelin-1, which is a more potent vasoconstrictor than endothelin-1 itself [6,26]. However, this hypothesis remains to be proved.

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