Matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 and 2 as potential biomarkers for gestational hypertension

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INTRODUCTION Gestational hypertension (GH) is a common disorder during pregnancy that can progress to preeclampsia and cause various subsequent fatal complications. A cluster of enzymes, called matrix metalloproteinases (MMPs), and its specific inhibitors, tissue inhibitors of metalloproteinases (TIMPs), have been reported to be involved in the pathophysiology of GH. The purpose of this study was to examine circulating levels of MMP-9, TIMP-1 and TIMP-2 in pregnant women who had GH and those who were normotensive.

METHODS In a case-control study, the total levels of MMP-9, TIMP-1 and TIMP-2 in the sera of 108 pregnant patients were evaluated using enzyme-linked immunosorbent assays. 54 patients with GH (test group) and 64 normotensive pregnant women (control group) were included in the study.

RESULTS While MMP-9 levels showed a high level of expression in the GH group ($p = 0.085$), TIMP-1 and TIMP-2 levels showed low levels of expression for the same. Weak positive correlations were found on correlation analysis between maternal age and TIMP-1 in the GH group ($r = 0.278$, $p < 0.05$), and between gestational age and TIMP-2 in the control group ($r = 0.318$, $p < 0.05$).

CONCLUSION Our findings suggest that MMP-9 may be involved in the pathophysiology of GH. It may be of value to further evaluate MMP-9 as a potential biomarker for predicting preeclampsia in pregnant women.

Keywords: biomarker, case-control, gestational hypertension, matrix metalloproteinase, tissue inhibitor of metalloproteinase
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INTRODUCTION

Changes in vascular remodelling, such as angiogenesis and trophoblastic invasion during normal pregnancy, are maintained by specific enzymes called matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs).[1-7] Gestational hypertension (GH) – a major precursor of preeclampsia (PE) that occurs in 6%–7% of pregnancies[8] – is characterised by abnormal proliferation and inadequate placental trophoblast invasion.[9,10] It has been hypothesised that an imbalance in the activities of MMPs and TIMPs[11] leads to various adverse events, such as poor foetoplacental perfusion, hypoxia, oxidative stress, inflammatory response, and the release of various cytokines and biochemical substances into the maternal circulation, resulting in high blood pressure[12,13] and proteinuria.[14]

Several types of biomarkers, much like MMPs and TIMPs, have been extensively studied to further understand the mechanisms of disease pathogenesis in PE.[15] We analysed the circulating levels of MMP-9, TIMP-1 and TIMP-2 in pregnant women with GH, and in those with normotensive pregnancies. This study is an adjunct to the growing body of research in this field and may lead to a better understanding of the mechanisms involved in the pathogenesis of GH.

METHODS

108 singleton pregnant women (age 18–40 years, gestation 20–40 weeks) attending antenatal clinics at the Kuala Lumpur Hospital and Serdang Hospital were included in the study. 54 patients had GH (test group) and 64 were normotensive (control). GH was defined according to the American College of Obstetricians and Gynecologists (ACOG) guidelines as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, without significant proteinuria after 20 weeks of gestation.[16,17] Normotensive pregnancy was defined as normal blood pressure without any complications throughout the pregnancy.

Informed consent was obtained from each patient prior to the study. Venous blood was collected in a serum separator tube (SST-II; Vacutainer tubes, Plymouth, UK). Serum was separated immediately at 3,000 rpm for 10 minutes at room temperature and stored at −80°C until laboratory analysis. Commercially available enzyme-linked immunosorbent assay kits were used for quantitative determination of MMP-9, TIMP-1 (Bender MedSystem, Vienna, Austria) and TIMP-2 (R&D Systems Europe, Abingdon, UK). The tests were run according to manufacturers' instructions.[18]

Although the estimated sample size was 20 patients when the significance level was set at 0.05 with a 95% confidence

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Table I. Clinical characteristics of the study participants.

Characteristic	Mean ± SD		p-value
	GH group (n = 54)	Normotensive group (n = 64)	
Median maternal age; IQR (yrs)	31; 27–36	28; 26–32	0.009*†
Median gestational age; IQR (wks)	31; 24–36	30; 24–35	0.525*
No. of nulliparous women (%)	21 (38.9)	28 (43.8)	0.595*
Systolic BP (mmHg)	141 ± 7	112 ± 11	0.001†‡
Diastolic BP (mmHg)	91 ± 7	66 ± 7	0.001†‡
Gestational age at delivery (wks)	37.6 ± 1.1	38.3 ± 1.1	0.001†‡
Birth weight (g)	2,833 ± 604	3,031 ± 387	0.041†‡

*Mann Whitney U test.†p < 0.05 was statistically significant.‡Independent t-test.
SD: standard deviation; GH: gestational hypertension; IQR: interquartile range; BP: blood pressure

Table II. Serum findings among the study participants.

Characteristic	Mean ± SD		p-value
	GH group (n = 54)	Normotensive group (n = 64)	
MMP-9 (ng/mL)	2,244.30 ± 1,176.64	1,875.97 ± 1,121.53	0.085*
TIMP-1 (ng/mL)	813.70 ± 217.66	766.08 ± 134.36	0.165*
TIMP-2 (ng/mL)	133.96 ± 46.38	130.93 ± 40.40	0.706*
Median MMP-9 /TIMP-1 ratio; IQR	2.74; 1.53–3.99	2.37; 1.27–3.36	0.204†
Median MMP-9 /TIMP-2 ratio; IQR	17.26; 10.17–23.93	11.99; 7.14–22.21	0.139†

* Normally distributed independent t-test. † Non-parametric Mann Whitney U test (not normally distributed).
SD: standard deviation; GH: gestational hypertension; MMP: matrix metalloproteinase; TIMP: tissue inhibitors of metalloproteinase; IQR: interquartile range

interval and 80% power of study (1-β = 0.80),[19,20] a higher number of patients were recruited for the study. All statistical analyses were performed using the Statistical Package for the Social Sciences, for Windows version 16 (SPSS Inc, Chicago, IL, USA). The continuous parameters for each group were compared using independent *t*-tests and Mann-Whitney U tests, and categorical parameters were compared using the chi-square test. Multivariate analysis with parametric Pearson's correlation analysis was used to determine the association between MMPs and TIMPs, and other clinical parameters, such as maternal age, gestational age and blood pressure.

RESULTS

Maternal age was significantly higher in patients in the GH group than the normotensive control group ($p < 0.05$; Table I). As expected, systolic and diastolic blood pressures were significantly higher in the GH group. The gestational age at delivery and mean birth weight were also lower in the GH group than in the control group. The GH group showed higher circulating MMP-9 levels and MMP-9/TIMP ratios than the control group. However, these differences did not reach statistical significance ($p > 0.05$). The TIMP-1 and TIMP-2 levels showed only low levels of expression in the GH group (Table II).

Further multivariate analyses conducted on the GH group showed a weak positive correlation ($r = 0.278$, $p = 0.047$) between TIMP-1 and maternal age, which subsequently led to a weak negative correlation ($r = -0.293$, $p = 0.036$) between MMP-9/TIMP-1 ratio and maternal age. The normotensive group showed weak positive correlation ($r = 0.318$, $p = 0.010$) between TIMP-2 and gestational age. No correlation was found between the biomarkers and blood pressure ($p > 0.05$).

DISCUSSION

Studies have shown the involvement of MMP-9 in cardiovascular diseases,[7,11] including GH[19,20] and PE.[21] We found a relatively increased MMP-9 level in GH group patients, consistent with the findings of Palei et al.[20] However, a few other studies have found no significant alteration in circulating MMP-9 levels,[19,21] which may possibly be due to normal placentation in pregnant women with GH.[21] A recent *in vivo* study found decreased expression of MMP-9 in preeclampic placental tissue when compared to normal placenta.[22] In PE, poor trophoblastic invasion and impaired implantation cause the release of cytokines and an inflammatory response.[5,8,12] Another *in vitro* study reported that cytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), increase MMP-9 production by stimulating cytotrophoblastic cells.[23] In PE, serum MMP-9 has been suggested to be associated with the inflammatory process due to increased levels of serum TNF receptor type 1.[21] It is possible that these events may alter the normal MMP/TIMP ratio. However, our findings suggested only a slight increase in MMP-9 levels in pregnant women with GH perhaps because the trophoblast invasion in GH was probably better than in PE. Net MMP activity has been found to be lower in older individuals compared to those younger in age, suggesting a decrease in extracellular matrix degradative capacity.[19] This is consistent with our findings, as TIMP-1 was found to correlate positively with increasing age in the GH group.

Our results showed a positive association between TIMP-2 and gestational age, which is also in line with previous *in vivo*[2] and *in vitro*[24] studies that reported that TIMP-1 and TIMP-2 were increased during late pregnancy, reflecting their role in vascular remodelling throughout pregnancy.[2] Although several MMPs and TIMPs have been found to be positively associated with hypertension,[7,25,26] our results indicated otherwise, suggesting that perhaps different pathophysiological mechanisms may be involved in GH.[19]

In conclusion, GH patients showed higher serum MMP-9 levels than normotensive pregnant women in our study. However, the study was not without limitations. The lack of statistically significant differences between the two groups may be due to our relatively small sample size. It is also possible that the results may have been skewed due to a multiracial study sample, as larger studies have found alterations in MMP-9 levels among the various broad ethnic groups.[18] Another probable factor that may have impact on the result is the type of sample used,[27] as artificial alterations in serum MMP-9 levels have been indicated in some studies.[28,29] Nonetheless, this study provides useful baseline information that has future implications. Further evaluation of these findings on a larger scale with a better-matched case-control study is warranted.

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