Matrix metalloproteinase-9 gene variants and pregnancy-induced hypertension in Egyptian women: a lack of association

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Background
Gestational hypertension (GH) is a common disorder during pregnancy that can progress to preeclampsia (PE). PE and its complications have become the leading cause of maternal and fetal morbidity and mortality worldwide. The development of PE is unpredictable and thus challenging to prevent and manage clinically. A cluster of enzymes, called matrix metalloproteinases (MMPs), have been reported to be involved in the pathophysiology of hypertensive states of pregnancy.

Aim
We aimed to investigate the association of two functional polymorphisms [−1562C/T and −90(CA)\textsubscript{13−25}] in the MMP-9 gene and their haplotypes with PE and/or GH in a group of Egyptian patients.

Methods
A total of 150 pregnant women; 50 healthy (control), 50 with GH, and 50 with PE were enrolled and genotyped for −1562C/T and −90(CA)\textsubscript{13−25} polymorphisms by PCR-RFLP and end-point PCR correspondingly.

Results
For both studied polymorphisms, no significant differences were found in genotype, allele, and haplotype frequencies when PE or GH groups were compared with control group.

Conclusions
Although MMP-9 −1562C/T and −90(CA)\textsubscript{13−25} polymorphisms and their haplotypes were not associated with either GH or PE Egyptian patients, the role of MMP-9 and its genetic variants cannot be ruled out in the pathophysiology of different hypertensive states of pregnancy.

Keywords:
Egypt, gestational hypertension, matrix metalloproteinase-9, preeclampsia

Introduction
Hypertensive disorders of pregnancy (HDP) are a significant health problem for women and their offspring worldwide. HDP are classified into (a) preeclampsia (PE)-eclampsia, (b) chronic hypertension (of any cause), (c) chronic hypertension with superimposed PE, and (d) gestational hypertension (GH) [1]. GH – a major precursor of PE – occurs in 5–10% of pregnancies, though its complications are less severe [2]. However, PE complicates 5–10% of all pregnancies worldwide [3]. PE and its complications have become a leading cause of maternal and fetal morbidity and mortality, responsible for ∼40% of births delivered at early gestation [4]. The development of PE is hardly predictable, therefore challenging to prevent and manage clinically [3]. Normally in early pregnancy, trophoblasts invade maternal vessels resulting in extracellular matrix remodeling, which leads to high uteroplacental vessel dispensability to accommodate the increased blood flow [5]. However, in PE, this trophoblastic invasion is reduced, causing inadequate modification of maternal spiral arteries and thus decreases the placental perfusion [6]. Matrix metalloproteases (MMPs) are a group of zinc-dependent proteolytic enzymes with various functions and tissue distribution [7]. MMPs target extracellular matrix components during development and morphogenesis [8]. Of them, MMP-2 and MMP-9 are involved in remodeling of placental and uterine arteries, and their abnormal expression have been described in HDP [9]. Gene polymorphisms can potentially affect the expression levels of the corresponding genes. Thus, the functional MMP-9 polymorphisms, −1562C/T substitution (rs3918242) and the microsatellite −90(CA)\textsubscript{13−25} (rs3222264), have been reported with many diseases, including cardiovascular diseases [10]. The aim of the present
study was to investigate the 2 functional polymorphisms [-90(CA)13–25 and -1562C/T] in the MMP-9 gene either alone or combined within haplotypes that are associated with GH and/or PE in a group of Egyptian patients from Kalyobia Governorate.

**Participants and methods**

**Participants**
This randomized case–controlled study was conducted on 150 pregnant females attending the Gynecology and Obstetrics Outpatient Clinic and Department, Benha University hospitals. They were divided into three groups: group I included 50 pregnant women with PE with mean age 26.24±2.55 years, group II included 50 pregnant women with GH with mean age 25.64±2.84 years, and group III included 50 apparently healthy age-matched pregnant women as a control group with a mean age of 25.48±3.15 years with no past or family history of GH or PE. Informed consent was obtained from each participant, and the study was approved by local ethics committee of Faculty of Medicine, Benha University. For group I, patients were diagnosed as PE after 20 weeks of gestation when the blood pressure was higher than 140/90 mmHg at two separate occasions, 6 h apart, along with significant proteinuria by dipstick reading of greater than 2+ on a voided random urine sample. For Group II, patients were diagnosed as GH after 20 weeks of gestation, when the blood pressure was higher than 140/90 mmHg at two separate occasions, 6 h apart. Any individual complicated by clinical chorioamnionitis or any infectious disorder or having urinary tract infection or any other cause of proteinuria was excluded from the study.

**Genotyping the MMP-9 polymorphisms**
Genomic DNA was extracted from peripheral blood leukocytes employing Gene Purelink Whole Blood Genomic DNA Purification Mini Kit QIAamp (QIAGEN, Hilden, Germany) according to the manufacture’s protocol. Two PCR reactions were performed separately using 2× DreamTaq Green PCR Master Mix (Thermo Scientific, Waltham, MA, USA), using the following primer sets: for MMP-9 (−1562C/T) (rs3918242), forward primer was 5’-GCCTGGCAC ATAGTTAGGCC-3’ and reverse primer was 3’-CTTCCTAGCCAGCCGCATC-5’. For MMP-9 (−90(CA)13–25) (rs3222264), forward primer was 5’-GACTTGGCAGTGAGACTGCGGGCA-3’ and reverse primer was 5’-GACCCCACCCTCTCTTGA CAGGCAA-3’. The reactions were performed in PikoReal 24 (Thermo Scientific), in the following steps: for rs3918242, initial denaturation at 95°C for 3 min; then 35 cycles of denaturation (95°C for 30 s), annealing (60°C for 30 s) and extension (72°C for 60 s); then a final extension step for 15 min at 72°C. For rs3222264, initial denaturation at 95°C for 3 min; then 35 cycles of denaturation (95°C for 30 s), annealing (65°C for 30 s), and extension (72°C for 60 s); and then a final extension step for 15 min at 72°C. The amplified product of the first SNP was subsequently digested by SphI restriction enzyme (Invitrogen, Carlsbad, CA, USA) at 37°C overnight. The enzyme digested the 435bp PCR product in the presence of T allele into 247bp and 188bp fragments, whereas the C allele remained un-cleaved, which was detected on 1% agarose gel (Fig. 1). In rs3222264, two sizes of tandem repeats were detected on 2% polyacrylamide gel mixed with urea to discriminate a high tandem repeat (H) (25 repeats) at 168bp from a low tandem repeat (L) (13 repeats) at 144bp (Fig. 2).

**Statistical analysis**
Statistical analysis was run on IBM statistical package for the social sciences program (SPSS; SPSS Inc., Chicago, IL, USA) version 20. Qualitative data were presented as frequency and percentage. χ²-Test or Fischer’s exact test.

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Fig. 1

Genotyping of the MMP-9 (rs3918242) by electrophoresis on 1% agarose gel. M: a 100bp marker, Lanes 1,7 TT genotype, Lanes 2,3,5,6 CC genotype and Lanes 4,8 CT genotype. MMP, matrix metalloproteinase.
were used to compare groups. Quantitative data are summarized as mean±SD. Comparisons between groups were done using Student’s t-test. Deviations from Hardy–Weinberg equilibrium expectations were determined using the χ²-test. Odds ratio and 95% confidence interval were calculated. The HaploView program (version 4.2) was applied to estimate the haplotypes and linkage disequilibrium, which uses the expectation maximization algorithm [11]. Tests are considered statistically significant if P value less than 0.05 at 95% confidence interval.

Results
The anthropometric measures along with the clinical and laboratory characteristics of the included groups are represented in Table 1. Healthy pregnant (control), GH and PE women were matched by age, gestational age, % primigravida, and hemoglobin concentration (P>0.05 each). As expected, PE and GH presented higher systolic and diastolic blood pressures compared with the control group (P<0.001 each). Significant proteinuria was found in patients with PE only when compared with both control and patients with GH (P<0.001 each). The distribution of genotypes for the two studied polymorphisms showed no deviation from Hardy–Weinberg equilibrium (P>0.05 each).

Results of genetic analysis of MMP-9 rs3918242
The genotype CC was the common genotype among the three studied groups (36/50 in control and 34/50 in GH and 38/50 in PE groups), with no significant differences (P>0.05). There were no significant differences among all groups regarding the allele frequency. The T allele frequency in the studied population (patients and controls) was 0.160, compared with the Global Minor Allele Frequency: 0.155 (Table 2). No statistically significant association was found between the MMP-9 rs3918242 genotypes and the mean age, primigravida, and mean hemoglobin concentration. Regarding gestational age, the CC genotype was significantly associated with patients with GH and PE when compared with the control group (P=0.008 and 0.002, respectively). However, for systolic and diastolic blood pressure, both CC and CT genotypes were significantly associated with patients with GH and PE when compared with control group (P<0.001 each) (data not shown).

Results of genetic analysis of MMP-9 rs3222264
The heterozygous genotype HL was the common genotype among the three studied groups (50/50 in control and 24/50 in GH and 20/50 in PE groups), with no significant differences (P>0.05). There were no significant differences among all groups regarding the allele frequency (P>0.05). There were no significant differences among all groups regarding the allele frequency (P>0.05) (Table 2). No statistically significant association was found between the MMP9 rs3222264 genotypes and the
**Table 2** Frequency distribution of matrix metalloproteinase-9 rs3918242 and rs3222264 genotypes and alleles among the studied groups

<table>
<thead>
<tr>
<th>Possible haplotypes</th>
<th>Control (n=50) (%)</th>
<th>GH (n=50) (%)</th>
<th>PE (n=50) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[n (%)]</td>
<td>[n (%)]</td>
<td>[n (%)]</td>
</tr>
<tr>
<td>rs3918242</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>36 (72)</td>
<td>34 (68)</td>
<td>38 (76)</td>
</tr>
<tr>
<td>CT</td>
<td>12 (24)</td>
<td>14 (28)</td>
<td>10 (20)</td>
</tr>
<tr>
<td>TT</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>C</td>
<td>84 (84)</td>
<td>82 (82)</td>
<td>86 (86)</td>
</tr>
<tr>
<td>T</td>
<td>16 (16)</td>
<td>18 (18)</td>
<td>14 (14)</td>
</tr>
<tr>
<td>rs3222264</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td>18 (36)</td>
<td>18 (36)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>HL</td>
<td>20 (40)</td>
<td>24 (48)</td>
<td>20 (40)</td>
</tr>
<tr>
<td>LL</td>
<td>12 (24)</td>
<td>8 (16)</td>
<td>16 (32)</td>
</tr>
<tr>
<td>H</td>
<td>56 (56)</td>
<td>60 (60)</td>
<td>48 (48)</td>
</tr>
<tr>
<td>L</td>
<td>44 (44)</td>
<td>40 (40)</td>
<td>52 (52)</td>
</tr>
</tbody>
</table>

CI, confidence interval; GH, gestational hypertension; OR, odds ratio; PE, preeclampsia. *P*, comparison between control and GH. §P, comparison between control and PE. ¶P, comparison between GH and PE.

Comparing the possible haplotypes of both MMP-9 studied variants among all groups did not show any significant association (*P*>0.05 each) (Table 3). However, the percentages of linkage disequilibrium between rs3918242 and rs3222264 were 71% in control group of normal pregnancy, 72% in patients with GH and PE when compared with the control group (*P*<0.001 each) (data not shown).

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**Discussion**

PE is a pregnancy-specific multisystem disease [12], which represents the second leading direct cause of maternal morbidity especially in developing countries [13]. Although exact pathogenesis of PE is not fully known, the inadequate placental perfusion that leads to extensive maternal endothelial dysfunction is suggested to be a major mechanism [8]. MMPs are proved to affect the vascular function and have a key role in vascular alterations occurs in PE and other cardiovascular diseases [14]. MMP-9 might be included in remodeling placental and uterine blood vessel and in controlling the vascular tone [15]. In the present study, we analyzed the genotype and allele frequencies for two functional MMP-9 polymorphisms [−90(CA)13–25 and −1562C/T] in pregnant women with PE and GH.

Serum MMP-9 level has been extensively studied in hypertensive states of pregnancy. It was found to be higher in patients with GH [16], but not with PE, when compared with normotensive pregnant women [17], suggesting that MMP-9 plays a role in the pathophysiology of GH and that HDP have different pathophysiological mechanisms. Moreover, the in-vitro genetic studies of MMP-9 showed that the ‘C’ to ‘T’ substitution at −1562 position is associated with increased MMP-9 expression [10]. Various studies revealed that the (CA)14 allele causes a 50% reduction in MMP-9 promoter activity as compared with the (CA)21 [18,19]. The elevated MMP9 concentrations reported to be associated with the −1562T allele might be essential for the development of an adequate maternal-fetal interface early in pregnancy by facilitating trophoblast apoptosis and degradation [20]. From our results, both MMP-9 −1562C/T and MMP-9 −90(CA)13–25 SNPs failed to show any significant association with either PE or GH. Our findings confirmed previous results
that did not find any significant association between MMP-9 −1562 (C>T) SNP and PE [21,22]. These results were also reported by Gong et al. [23] in their meta-analysis, which revealed no evidence for significant association between MMP-9 −1562C/T polymorphism and risk of PE. Although we did not find any association between the minor allele T of MMP-9 −1562C/T polymorphism and neither GH nor PE, previous studies reported a significant association of the T allele with the risk of GH but not PE, which might explain the higher plasma MMP-9 levels previously reported in GH compared with HP [24,25]. However, Coolman et al. [20] found a lower prevalence of the rare T allele in women with PE (odds ratio: 0.48, 95% confidence interval: 0.25–0.90), and they suggested that the MMP-9 −1562T allele is associated with a reduced risk of PE and therefore may protect against maladaptation of the spiral arteries and decreased decidual degradation. Regarding genotypes, neither CT nor TT genotype was associated with the studied groups. This was on the contrary of previous findings by Rahimi et al. [26] who observed a significantly higher frequency of CT genotype in both mild and severe preeclamptic women compared with controls. They found that the presence of CT +TT genotype was significantly higher in early-onset rather than late-onset PE. Moreover, the presence of CT+TT genotype increased the risk of severe PE by 2.37-fold, which led them to suggest the role of MMP-9 variant as a useful biomarker of susceptibility to severe PE and early-onset severe PE [26]. From our results, we could not detect any significant association of MMP-9 −90(CA)13–25 with GH or PE. However, in a previous study, no significant differences were found in genotype and allele distributions when PE or GH groups were compared with control group [24]. Although Palei et al. [25] had we found increased MMP-9 levels in patients with GH with the LH genotype for the −90(CA)13–25 polymorphism. In our haplotype analysis, we could not find any significant association between any of the possible four haplotypes and GH or PE. These results confirmed the findings of Palei et al. [24] who found no significant differences in overall distributions of haplotype frequencies when the GH or the PE group was compared with the HP group. However, in another study although no differences were found when haplotypes were compared between PE and healthy pregnant women, the distribution of MMP-9 TH haplotype was more commonly found in the GH group than in the healthy pregnant women [25].

In conclusion, our limited study on a small group of Egyptian population demonstrated that MMP-9 −1562C/T and −90(CA)13–25 polymorphisms in addition to their haplotypes did not show significant deviation in patients with GH or PE compared with normotensive pregnant women controls. More studies with larger sample size and multiple subgroups are needed to further explore the association between them. This will help to predict the risk of PE and take measures to avoid the maternal and fetal morbidity and mortality.
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N.A.A., E.R.A., and A.W.A.M. designed the study and defined the intellectual content; O.S.E. and R.A.K. searched the literature and performed clinical and experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, and manuscript editing; N.A.A., E.R.A., A.W.A.M., O.S.E., and R.A.K. reviewed the manuscript.

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Conflicts of interest
There are no conflicts of interest.

References