Role of matrix metalloproteinase-9 expression in the pathogenesis of early-onset preeclampsia: a case–control study

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Context
Preeclampsia (PE) is a pregnancy disorder characterized by impaired invasiveness of the placenta in which matrix metalloproteinases (MMPs), especially MMP-9, play an important role.

Aim
To investigate the expression of MMP-9 in the placenta of patients with early-onset preeclampsia (EOPE) compared with controls.

Setting and design
The study included 50 pregnant women programmed for termination of pregnancy at a gestational age of 28–36 weeks. Women were divided into two groups: severe EOPE group ($n = 30$) and healthy pregnant control group ($n = 20$).

Patients and methods
A tissue was cut off from the center of the placenta, and the tissue was preserved for subsequent MMP-9 expression by real-time quantitative PCR.

Results
Neonatal and placental weights were significantly higher in the control group ($P < 0.001$). MMP-9 mRNA was significantly higher in the placentae of the control group compared with that of the preeclampsia group ($P < 0.001$).

Conclusion
MMP-9 expression in the placenta was significantly downregulated in patients with EOPE compared with normal pregnant women, suggesting their important role in trophoblast invasion.

Keywords:
matrix metalloproteinase, preeclampsia, pregnancy

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Introduction

Preeclampsia (PE) is a disorder characterized by the new onset of hypertension and proteinuria in previously normotensive women. PE causes morbidity and mortality in both mother and fetus and is one of the most serious problems in obstetrics worldwide, affecting 2–8% of pregnancies [1].

The pathophysiology of PE is still vague but the impaired invasiveness of the trophoblast cells of the placenta is the main factor [2]. The invasive property of trophoblast cells depends on the production of matrix metalloproteinases (MMPs) [3].

MMP-9 is one of the important members of MMP family. MMP-9 can degrade type IV collagen and is directly involved in the process of embryo invasion and implantation [4,5]. A dysregulated secretion of MMP-9 could interfere the physiological trophoblast invasion. Thus, we measured its expression in placentae of PE women relative to normal pregnant women.

Patients and methods

Study design

This is a case–control study. The study protocol was registered in clinicaltrials.gov: NCT03258125.

Study setting and duration

The study has been conducted through collaboration between Medical Physiology Department and Obstetrics and Gynaecology Department in Assiut University during the period between January and December 2019.

Ethical considerations

The study protocol was approved by the Ethical Committee at Faculty of Medicine, Assiut University,

and all participants signed an informed consent, with approval no: IRB17200134.

Study participants

The study included 50 pregnant women scheduled for termination of pregnancy by vaginal delivery or cesarean section at a gestational age of 28–36 weeks. Their age range was 18–40 years.

The women were divided into two groups:
(1) Control group (n = 20).
(2) Severe early-onset preeclampsia (EOPE) group (*n* = 30).

The diagnosis of PE was based on the modified American College of Obstetricians and Gynaecologists (ACOG) criteria 2019, which define PE as a pregnancy complicated disorder with new onset of hypertension (blood pressure ≥140/90 mmHg) and proteinuria (urine dipstick ≥+2 protein or ≥300 mg protein/24-h urine sample) after 20 weeks of gestation. EOPE is PE starting before 34 weeks of gestation. Severe PE is defined as blood pressure more than or equal to 160/110 mmHg and urine dipstick more than or equal to +3 protein or 24-h urine protein more than or equal to 2000 mg [1].

Exclusion criteria

Diabetes mellitus, chronic hypertension, nephropathy, acute or chronic infectious diseases or other chronic illness, twin pregnancy, and anti-phospholipid antibody syndrome were the exclusion criteria.

PE complicated with eclampsia or hemolysis, elevated liver enzymes, low platelets count (HELLP) syndrome, patients with cancer, and smoking were excluded.

Procedures

(1) Before delivery, all women were subjected to the following:
   (a) History taking for detection of age of the patients, parity, and gestational age in weeks.
   (b) Calculation of BMI [weight in kg/(height in m2)].
   (c) Measurement of blood pressure (systolic and diastolic).
   (d) Detection of urinary protein by urine dipstick test.
(2) After delivery, the following measures were taken:
   (a) Measurement of total weight of the placenta in grams.
   (b) Measurement of neonatal weight in grams.
   (c) A placental tissue was cut off from the center of maternal side, avoiding areas of infarction, bleeding, or calcification. The tissue was preserved in RNAlater solution to protect the RNA content. The tissue was placed in at least 10 volumes of RNAlater (RNA Stabilization Reagent) or ~10 μl of reagent per 1 mg of tissue.
   (d) Assessment of MMP-9 in the placentae of both controls and patients by real-time PCR.

The relative expression levels of miRNA-425 and MMP-9 versus the housekeeping genes (RNU6 and B actin, respectively) were calculated using the equation of $2^{-\Delta\Delta ct}$.

$^{\Delta}$CT was calculated by subtracting the corresponding CT value of housekeeping gene from the CT value of the target.
(i) $^{\Delta\Delta}$CT for patients was calculated by subtracting the ΔCT of the PE samples from that of the mean of the control group, and $^{\Delta\Delta}$CT for control was calculated by subtracting the ΔCT of the control samples from that of the mean of the control group.

Statistical analysis

Data were analyzed using Statistics Package for Social Sciences (SPSS), version 20 (SPSS Inc., Chicago, Illinois, USA). Normality test (Shapiro–Wilk test) was performed, and data (age, BMI, gestational age, placental and neonatal weights) were normally distributed. In contrast, data for MMP-9 were not normally distributed. Continuous data were expressed as mean ± SD. Differences between the two groups were detected using independent samples t test for parametric data and Mann–Whitney test for nonparametric data. Nominal data were expressed as percentage. The differences between the two groups were detected using $\chi 2$ test. Pearson's correlation between placental and neonatal weight was used.

Results

Basic characteristics of the studied groups

Tables 1 and 2 show the differences between both groups regarding basic characteristics and pregnancy outcome. Table 1 shows that there was no statistically significant difference between the two groups regarding their age, BMI, or gestational age. However, all of women in the control group were multigravida compared with 46.7% in PE group, and this was statistically significant. Table 2 shows that there was no significant difference between the two groups regarding the mode of delivery. However, both placental and neonatal

weights were significantly higher in the control group compared with the PE group. There was a positive significant correlation between placental and neonatal weights (Table 3; Fig 1).

Table 1: Differences between studied groups regarding basic characteristics

Variables	Control group (*n*=20)	PE group (*n*=30)	*P*
Age (years)	26.65±5.412	26.33±6.483	0.853 (NS)
BMI (kg/m2)	29.12±3.952	29.43±4.26	0.789 (NS)
Parity "*n* (%)"			
Primigravida	0 (0%)	16 (53.3%)	<0.001*
Multigravida	20 (100%)	14 (46.7%)	
Gestational age (weeks)	35.75±0.716	34.37±2.282	0.072 (NS)

Data are presented as mean±SD or n (%). NS, nonsignificant; PE, preeclampsia. Student's t test for age, BMI, and gestational age and χ^2 test for parity were used. *Significant P value.

Table 2: Pregnancy outcomes of studied groups

Variables	Control group (*n*=20)	PE group (*n*=30)	*P*
Mode of delivery "*n* (%)"			
VD	1 (5%)	4 (13.3%)	0.336 (NS)
CS	19 (95%)	26 (84.75)	
Placental weight (g)	575.3±112.5	452.3±137.3	0.001*
Neonatal weight (g)	3115±436.8	2252±612.3	<0.001*

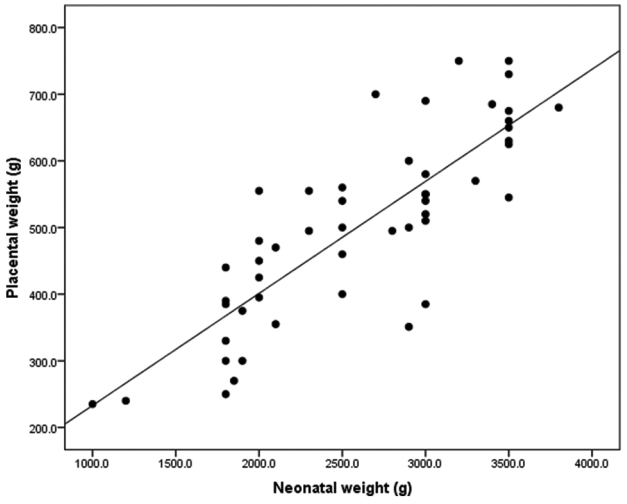
Data were presented as mean±SD or n (%). CS, cesarean section; NS, nonsignificant; PE, preeclampsia; VD, vaginal delivery. Student's t test for placental and neonatal weight and χ2 test for mode of delivery were used. *Significant P value.

Table 3: Correlation between placental weight and neonatal weight in studied groups (*n*=50)

Parameters	Correlation Coefficient (*r*)	*P*
Placental weight/Neonatal weight	0.822	<0.001*

Pearson's correlation was used to correlate placental weight and neonatal weight. *Significant *P*

Figure 1



Pearson's correlation between placental weight and neonatal weight in studied groups. Placental and neonatal weights show a positive significant correlation.

Relative expression of matrix metalloproteinase-9 in the placenta

Table 4 shows that MMP-9 mRNA was significantly higher in the placentae of the control group compared with that of the PE group (P < 0.001) (Fig 2).

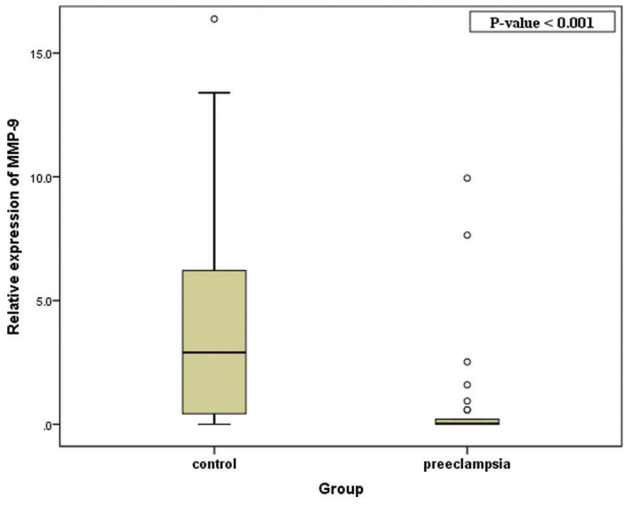
Discussion

MMP-9 is one of the important members of MMP family. MMP-9 can degrade the main ingredient in ECM-type IV collagen and is directly involved in the process of chorionic epithelium cells invading endometrium and embryo implantation [5].

In this study, placental MMP-9 mRNA expression was significantly downregulated in the PE group compared with the controls. The downregulation of MMP-9 expressions may inhibit the implantation and invasion ability of placenta, leading to the development of EOPE. This result is consistent with Plaks *et al*. [6] and Arora *et al*. [7], who found that MMP-9 mRNA expression was reduced in both plasma and placentae of patients with PE as compared with the controls. In addition, expression of MMP-2, MMP-8, MMP-9, and MMP-11 was lower in placentae of women with PE compared with normal women [8].

In contrast, in the study by Yan *et al*. [9], patients with EOPE had a significantly elevated serum MMP-9 level compared with normal pregnant women, suggesting a role of MMP-9 in the breakdown of vascular collagen that possibly predisposes to edema and proteinuria observed in these patients with PE. Moreover, in the

Figure 2



Box plots of the relative expression of MMP-9 mRNA in the placentae of the patients and controls. Box plots show the median and interquartile range. Mann–Whitney *U* test was used for statistical analysis. MMP-9, matrix metalloproteinase-9.

Table 4: Relative expression of placental matrix metalloproteinase-9 in the studied groups ($2^{-\Delta\Delta Ct}$)

Studied Gene		Control group (*n*=20)	PE group (*n*=30)	*P*
MMP-9/β-actin	Median (min-max)	2.90 (0.0026-16.38)	0.028 (0.0005-9.945)	<0.001*

Data are expressed as Median (min-max). MMP-9, matrix metalloproteinase-9; PE, preeclampsia. Mann–Whitney *U* test was used.
*Significant P value.

study by Karampas *et al*. [10], there was no difference in the level of MMP-9 between normal and PE women. Moreover, in another study, 14 biomarkers for PE, including MMP-2 and MMP-9, were measured in urine samples. MMP-2 and MMP-9 urine concentrations were significantly higher in women with severe PE compared with normal pregnant women and continued 6–8 weeks after delivery [11]. The controversy between these studies and our study might be explained by the different tissues used; they measured MMP-9 in serum and urine, whereas we measured it in the placenta.

We also compared the placental weight and the neonatal weight between the two studied groups. There was a significant decrease in the placental weights of EOPE group compared with those of control group. Moreover, the weights of newborns in EOPE group were lower than those of the control group. These results suggest that patients with EOPE may have development a disorder with placenta, which directly causes the limited growth of fetus. These results are consistent with Panti *et al*. [12], in which placental and neonatal weight correlated positively, and the ratio of the placental and neonatal weights at term decreases with advancing gestational age.

In conclusion, MMP-9 expression in the placenta was significantly downregulated in patients with EOPE compared with normal pregnant women, suggesting its important role in trophoblast invasion.

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Conflicts of interest
None declared.

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