

Are there any differences between preeclamptic and healthy pregnant women for serum and placental levels of milk fat globule epidermal growth factor-8 and suppressor of cytokine signaling-3?

Abstract

Purpose: Preeclampsia (PE) is closely associated with systemic inflammatory response. Milk Fat Globule Epidermal Growth Factor-8 (MFG-E8), an endometrial epithelial protein, regulates inflammation and apoptosis. Suppressor of cytokine signaling-3 (SOCS-3) plays role on prevention of inflammation. We evaluated the serum and placenta levels of MFG-E8 and SOCS-3 in preeclampsia and healthy pregnant women.

Methods: The study population was consisted of 40 preeclampsia and 50 healthy, gestational-age matched women with no history of preeclampsia. Prepartum serum and postpartum placental tissue samples were collected. Serum and placenta MFG-E8 and SOCS-3 levels were analysed with enzyme-linked immuno sorbent assay method.

Results: In preeclamptic group, serum MFG-E8 levels raised and showed statistically significant difference with respect to control group ($p < 0.01$). Placental SOCS-3 levels were significantly high in preeclamptic group compared to that in control group. Serum MFG-E8 levels showed positive correlation with both of blood pressure and proteinuria level.

Conclusions: In our study, serum MFG-E8 level showed close relation with blood pressure and proteinuria. Increased placental SOCS-3 levels might be the result of compensatory angiogenic and anti-inflammatory response in PE. New studies with expanded populations and genetic evaluations are needed to discuss our results.

Keywords: MFG-E8, SOCS-3, Preeclampsia, Placenta

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Introduction

Preeclampsia (PE) is a placenta-induced inflammatory disease associated with maternal and fetal morbidity and mortality.^{1,2} The mechanisms underlying PE is still unclear but generally accepted that in PE, the factors released from placenta into the maternal circulation induce inflammation.³ Significantly elevated circulating factors in PE include cytokines and antiangiogenic factors and these factors modulate trophoblast invasion.⁴ The hypothesis produced by Ahmed and Ramma suggested that preeclampsia might be result of the imbalance between new-onset inflammatory state and endogenous protective pathways during pregnancy.⁵ Milk Fat Globule Epidermal Growth Factor-8 (MFG-E8), a novel endometrial epithelial protein, found to have multiple biological functions against inflammation.^{6,7} MFG-E8 binds to apoptotic cells, enhances the phagocytosis of apoptotic cells by macrophages and provides an anti-inflammatory function.^{8,9} Topical recombinant MFG-E8 treatment induces resolution of wound inflammation, and improvement in angiogenesis.¹⁰ Suppressor of cytokine signaling-3 (SOCS-3) plays an important role in negative regulation of inflammatory response.¹¹ Decreased serum and placental SOCS-3 levels in preeclampsia may account for the increased inflammatory response in PE.¹²⁻¹⁴ We hypothesize that both of MFG-E8 and SOCS3 levels may decrease or increase either being result or reason of PE. And we compared the serum and placental levels of MFG-E8 and SOCS-3 between preeclampsia and gestational-age matched healthy pregnant women.

Materials and methods

Study population

This prospective, cross-sectional study was conducted with 90 pregnant women who were hospitalized at Firat University Hospital, Department of Gynecology and Obstetrics between December 2013 and December 2015 after local ethics committee approval (Decision No: 06/2013). The study population was consisted of 40 preeclampsia pregnant women (study group) and 50 healthy, gestational age matched pregnant women without previous history of PE (control group). Diagnosis of PE was defined as follows: New onset hypertension (>140 mmHg systolic or >90 mmHg diastolic) and proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L [$^{+2}$] on dipstick testing) after 20 weeks gestation.¹⁵ Proteinuria was recorded according to the grading of dipstick. Exclusion criteria included diabetes mellitus, chronic hypertension, severe heart, liver or renal dysfunction, other obstetric or medical syndromes and history of smoking, drinking, drug abuse or mental illness. To avoid clinical phenotypic differences in preeclampsia patients, patients complicated with HELLP syndrome (hemolysis, elevated liver enzyme and low platelet count) were excluded. Signed informed consent was obtained at the time of enrollment.

Enzyme-linked immunosorbent assay (ELISA)

Blood: Prepartum five milliliters peripheral venous blood samples were taken from preeclampsia pregnant women during obstetric unit

admission. The blood samples of gestational age-matched control group were taken during examination at out-patient clinic. After centrifugation for 15 minutes at 2500r/min at 4°C, the serum samples were stored at -80°C until further analysis. Serum *MFG-E8* (Boster Biological Technology, Pleasanton, CA, catalog No: EK1201) and *SOCS-3* (Sunred Biological Technology, Shanghai, China, catalog no: 201-12-0658) levels were analysed with ELISA method according to manufacturer's instructions. The detection limit (sensitivity) of the assay was 20ng/mL for MFG-E8 and 9ng/mL for SOCS-3. All assays were carried out by personnel who were blinded to the study.

Placental tissue collection and processing

Postpartum placental tissue samples were collected from the placental villi area within 20 minutes after delivery. Approximately 0.350gr placental tissues were cut out and individually rinsed in 3.5mL phosphate buffer saline (PBS) to wash off maternal and fetal blood. Homogenate preparation was done on ice with intermittent vortexing. Homogenates were centrifuged at 5000×g for 5 minutes and then the supernatants were separated and allowed to stand at -80°C until run time. Microparticles analysis (LOWRY method) was used to calculate placental protein concentration values in terms of pictogram / gram protein for application of appropriate amount of sample to ELISA. Architect 4000 (Abbott, USA) auto analyzer was used for micro protein analysis. Placental MFG-E8 (Booster Biological Technology, Pleasanton, CA, catalog No: EK1201) and placental SOCS-3 (Sun red Biological Technology, Shanghai, China, catalog no: 201-12-0658) levels were analysed with ELISA method. The detection limit

(sensitivity) of the assay was 8pg/mL for MFG-E8 and 50pg/mL for SOCS-3. All assays were carried out by personnel who were blinded to the study. Placental MFG-E8 and SOCS-3 concentrations have been normalized for 1 mg of total protein content.

Results

The mean age and body mass index of all women in the study were 28.2±6 years and 26.6±3.4kg/m² respectively. Delivery week, birth weight and apgar1 score were statistically lower in preeclamptic group compared to those in control group (Table-1). Serum and placental tissue levels of all parameters were presented in Table 2. In preeclamptic group, serum MFG-E8 (p<0.01) and OPG (p<0.05) levels raised and showed statistically significant difference with respect to control group. There was no significant difference between groups for serum levels of SOCS-3. Placental SOCS-3 levels were significantly high in preeclamptic group compared to that in control group. There was no significant difference between groups for placental levels of MFG-E8. Serum MFG-E8 levels showed positive correlation with both of blood pressure (R=0.23, p=0.03) and proteinuria level (R=0.29, p<0.01). In regression analysis, only serum MFG-E8 levels showed influence on blood pressure and this influence was minimal (OR=0.3, 95% CI=0.001- 0.005, p<0.01). Serum MFG-E8 levels showed negative correlation with both of apgar1 score (R=-0.24, p=0.02) and birth weight (R=-0.25, p=0.01). Serum MFG-E8 levels showed minimal influence on birth weight (OR=0.24, 95%CI= 0.009- 0.09, p=0.02).

Table 1 Clinical characteristics of all women in the study

	Control (N=50)	Preeclampsia (N=40)	P Value
Age (Years)	27.7±0.8	29.4±1	0.2
Body mass index (kg/m ²)	25.1±0.2	28.9±0.6	<0.01
Gravida (Number)	3.2±0.3	2.1±0.2	0.01
Delivery week	38±0.2	34±0.5	<0.01
Apgar1 score	8±0.2	7±0.3	<0.01
Neonate birth weight (gram)	3150±76	2153±133	<0.01

Note: Values are presented as mean±SEM.

Table 2 Serum and placental levels of all women in the study

	Control (N=50)	Preeclampsia (N=40)	P value
Serum MFG-E8 (ng/mL)	2680±375	6664±3106	<0.01
Placental MFG-E8 (pg/gr protein)	3647355±248075	4704684±849396	0.2
Serum SOCS-3 (ng/mL)	12±0.5	12±0.3	0.8
Placental SOCS-3 (pg/gr protein)	18334±6822	73694±22964	0.02

Note: Values are presented as mean±SEM.

MFG-E8: Milk Fat Globule Epidermal Growth Factor-8;

SOCS-3: Suppressor of Cytokine Signaling-3

Discussion

To the best of our knowledge, our study is the first investigating serum and placental MFG-E8 levels in PE. We observed significantly increased serum MFG-E8 levels and increased placental SOCS-3 levels in preeclamptic pregnant women compared to those in control group. We also observed minimal influence of serum MFG-E8 levels on maternal blood pressure and birth weight of neonate. Our study has the following limitations;

- We could not investigate the inflammatory condition of placenta with histopathological methods.
- The delivery week and BMI of control group could not be matched with preeclamptic group.

In our knowledge, there is no study performed previously on the relation between MFG-E8 and PE. So we will discuss our results on the basis of anti-inflammatory effect of MFG-E8. MFG-E8 plays role in the phagocytosis of apoptotic cells.^{16, 17} The serum level of MFG-E8 was found to be higher in SLE patients as compared to very low level in healthy controls.¹⁸ But patients with rheumatoid arthritis showed lower serum concentrations of MFG-E8 compared with healthy controls.⁹ It was reported that administration of recombinant MFG-E8 suppressed the ischemia-reperfusion induced inflammation in mouse.¹⁹ In an atherosclerosis study, investigators suggest that the lack of MFG-E8 leads to acceleration of disease development by accumulation of apoptotic cells due to suppression of protective antiinflammatory responses.²⁰ In an animal inflammatory bowel

disease model, the colons of animals receiving human recombinant MFG-E8 showed distinct decreament in inflammation markers.²¹ Researchers observed three fold increament in expression of MFG-E8 during implantation period.^{22, 23} Schmitz et al.²⁴ observed decreased trophoblast attachment by blocking of MFG-E8 on endometrial cell line in an invitro study. Bocca et al.⁶ observed expression of MFG-E8 on human placenta at all trimesters of gestation. In our study we observed the serum MFG-E8 levels of preeclamptic pregnant women higher than that of healthy pregnant women. But we did not observe any difference between preeclamptic and healthy pregnant women for the placental levels of MFG-E8. This difference may arise from another tissue source of MFG-E8.

Researchers suggested that SOCS-3 gene expression could improve anti-inflammatory response by IL-10 production in placental trophoblasts.¹¹ Guo et al.²⁵ studied serum SOCS-3 levels in 40 preeclamptic and 40 normotensive pregnant. They observed significantly decreased SOCS-3 levels in preeclamptic women compared to that in normotensive controls. Ozkan et al.¹² observed negative correlation between maternal plasma SOCS-3 levels and severity of PE. Wang et al.¹³ reported reduced SOCS-3 expression in leukocyte and vessel endothelium of preeclamptic women compared to those in normotensive pregnant. Zhao et al.¹⁴ evaluated decreased placental SOCS-3 expression in preeclamptic women. In our study; we did not observe difference between serum SOCS-3 levels of preeclamptic and normotensive controls, but placental SOCS-3 level of preeclamptic women was significantly higher than that of controls. In conclusion; serum MFG-E8 levels showed mild correlation with blood pressure and proteinuria in PE. Increased serum MFG-E8 levels of preeclamptic women may point to induced angiogenic and apoptotic factor production. And increased placental SOCS-3 levels might be the result of anti-inflammatory compensation in preeclamptic placenta. It is not clear with our study whether these findings are result or reason in PE pathogenesis. Of course new studies are needed to discuss our findings.

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Conflicts of interest

No conflict of interest.

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