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Serum levels of growth arrest specific protein 6 are increased in preeclampsia

Holger Stepan [a,1], Judit Richter [b,c,*,1], Karoline Kley [b], Susan Kralisch [b,c], Alexander Jank [a], Wiebke Schaarschmidt [a], Thomas Ebert [b], Ulrike Lössner [b,c], Beate Jessnitzer [b], Jürgen Kratzsch [d], Matthias Blüher [b], Michael Stumvoll [b], Mathias Fasshauer [b,c]

[a] University of Leipzig, Department of Obstetrics, 04103 Leipzig, Germany
[b] University of Leipzig, Department of Endocrinology and Nephrology, 04103 Leipzig, Germany
[c] Leipzig University Medical Center, IFB AdiposityDiseases, 04103 Leipzig, Germany
[d] University of Leipzig, Institute of Laboratory Medicine, 04103 Leipzig, Germany

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ABSTRACT

Preeclampsia (PE) contributes to maternal and fetal morbidity and mortality worldwide. Moreover, it is associated with an increased future metabolic and cardiovascular risk for mother and newborn. Recently, growth arrest specific protein (Gas) 6 has been introduced as a novel metabolic risk factor with anti-angiogenic, pro-atherogenic, and pro-adipogenic properties. In the current study, we investigated serum concentrations of Gas6 in patients with PE (n = 51) as compared to healthy, age-matched controls (n = 51) during and 6 months after pregnancy. Furthermore, association of Gas6 with markers of renal function, glucose and lipid metabolism, as well as inflammation, was assessed in all individuals. Median maternal Gas6 serum levels adjusted for body mass index and gestational age at blood sampling were significantly increased in PE patients (5.7 μg/l) as compared to healthy, age-matched pregnant women (4.6 μg/l) ($p<0.05$). Furthermore, Gas6 concentrations positively correlated with blood pressure, creatinine, free fatty acids, C-reactive protein, leptin, and adiponectin during pregnancy. Moreover, leptin and adiponectin remained independently associated with Gas6 levels in multivariate analysis. Gas6 serum levels 6 months after pregnancy were not significantly different between former PE and control patients. Taken together, maternal Gas6 serum concentrations are significantly increased in PE during pregnancy. Furthermore, the adipokines leptin and adiponectin are independent predictors of circulating Gas6 in pregnant women.

1. Introduction

Preeclampsia (PE) represents a serious complication during pregnancy and affects approximately 2% to 8% of all pregnancies. New-onset of hypertension and proteinuria associated with multisystem abnormalities during the second half of pregnancy is the characteristic clinical feature of PE. Investigations on the pathogenesis of PE are of great interest, since PE is associated with an increased risk for neonatal and maternal morbidity and mortality, as well as future development of vascular and metabolic disease [1].

The pathophysiology of PE has been better elucidated in recent years. Thus, dysregulation of angiogenic and anti-angiogenic factors including endoglin and soluble fms-like tyrosine kinase (sFlt) 1 contributes to the disease [2–6]. Furthermore, PE shares various risk factors with the metabolic syndrome including obesity, insulin resistance, and dyslipidemia. Interestingly, various metabolic risk factors have recently been described to potentially contribute to the pathogenesis of PE. Among those, increased concentrations of the appetite-suppressive adipokine leptin have been found in PE which precede the clinical onset of the disease [7]. Furthermore, hyperleptinemia might act as a compensatory response to increase nutrient delivery to the underperfused placenta [8].

Recently, circulating growth arrest specific protein (Gas) 6 has been introduced as a novel metabolic risk factor with anti-angiogenic, pro-atherogenic, and pro-adipogenic effects. Thus, Gas6 inhibited ligand-dependent activation of vascular endothelial growth factor (VEGF) receptor 2 and, thereby, the angiogenic program in endothelial cells through its receptor Axl [9]. Furthermore, Gas6 suppressed endothelial cell morphogenesis and VEGF-A-dependent vascularization [9]. Moreover, Gas6 is expressed in macrophages, endothelial cells, and smooth muscle cells and the expression increases with the severity of atherosclerosis [10]. In addition, studies in Gas6/ApoE-deficient mice suggest that Gas6 promotes instability of the atherosclerotic plaque due to increased endothelial cell activation and leukocyte recruitment [10]. Gas6 also has pro-adipogenic properties and is implicated in adipose tissue development [11].

In contrast to established anti-angiogenic factors including sFlt1 and endoglin, as well as metabolic risk factors including leptin, the role of Gas6 in pregnancy and its possible involvement in the pathogenesis of

* Corresponding author at: Liebigstraße 21, 04103 Leipzig, Germany. Tel.: +49 341 9713318; fax: +49 341 9713309.
E-mail address: judit.richter@medizin.uni-leipzig.de (J. Richter).
[1] These authors equally contributed to this work.

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PE have not been elucidated so far. Therefore, we investigated for the first time in the current study whether the levels of maternal Gas6 are increased during and 6 months after pregnancies complicated by PE. For this purpose, we determined circulating Gas6 in 51 women with PE and 51 normotensive, age-matched controls during and after pregnancy and correlated Gas6 serum levels with biochemical and clinical markers of renal function, glucose and lipid metabolism, as well as inflammation.

2. Methods

2.1. Study population

For the current study, 102 women with PE (n = 51) and controls (n = 51) were recruited from the Department of Obstetrics, University of Leipzig. At the time of blood sampling, none of the women was in labor. Presence of PE was defined as the gestational blood pressure elevation > 140 mm Hg systolic or > 90 mm Hg diastolic accompanied by proteinuria in women who were normotensive before 20 weeks of gestation according to [12]. Body mass index (BMI) was calculated as weight before pregnancy divided by squared height. The BMI of the patients ranged from 16.9 to 43.0 kg/m^2 and the age from 18 to 40 years. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously described [13]. Renal function was assessed as glomerular filtration rate (GFR) estimated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [14]. Patients with diabetes mellitus or renal diseases were excluded from the study. Since the hallmarks of PE have resolved within six months [15,16] after delivery, fasting blood samples from 89 out of 102 women including 44 former patients with PE and 45 former controls were collected at that time point. Again, all women underwent a clinical examination. The study protocol was approved by the local Ethics Committee. All patients gave a written informed consent before taking part in the study.

2.2. Assays

All blood samples were obtained after an overnight fast. Immediately after sampling, serum was separated by centrifugation at 4000 *g* for 10 min and frozen at −80 °C. Circulating Gas6 (R&D Systems, Minneapolis, USA), adiponectin (Mediagnost, Reutlingen, Germany), and leptin (Mediagnost, Reutlingen, Germany) were determined with enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions. Serum creatinine, fasting glucose (FG), fasting insulin (FI), free fatty acids (FFA), total, high density lipoprotein (HDL), and low density lipoprotein (LDL) cholesterol, triglycerides (TG), and C-reactive protein (CRP) were measured by standard laboratory methods in a certified laboratory.

2.3. Statistical analysis

All statistical analyses were performed with SPSS software version 20.0 (IBM, Armonk, USA). Differences in circulating levels of Gas6 between control and PE patients during pregnancy or after pregnancy were assessed by Mann–Whitney-*U* test or unpaired Student's *t*-test followed by Bonferroni adjustment for multiple testing. Before undergoing these tests, all variables were adjusted for BMI and gestational age at blood sampling during pregnancy and for BMI and time from delivery. In more detail, a general linear model analysis was performed for each parameter including BMI and gestational age at blood sampling or BMI and time from delivery as covariates. Non-standardized residuals were then taken forward as a new dependent variable to determine subgroup (control, PE) differences by Mann–Whitney-*U* test or unpaired Student's *t*-test. Changes in circulating Gas6 within the same individual during and after pregnancy were identified using Wilcoxon signed-rank test for related samples or paired Student's *t*-test followed by Bonferroni adjustment for multiple testing. A p-value < 0.05 in the Results section indicates that statistical significance was obtained in both Mann–Whitney-*U* test and unpaired Student's *t*-test or Wilcoxon signed-rank test for related samples and paired Student's *t*-test. Univariate correlations were performed using Spearman's rank correlation method. To adjust the effects of covariates and identify independent relationships, multivariate linear regression analyses were performed. Distribution was tested for normality using Shapiro–Wilk W test and non-normally distributed parameters were logarithmically (lg) transformed before multivariate analyses. A p-value of <0.05 was considered as statistically significant in all analyses.

3. Results

3.1. Gas6 serum levels are increased in PE patients

Table 1 summarizes the clinical characteristics of the subgroups (Control, PE) studied. Median (interquartile range) maternal serum Gas6 concentrations were significantly elevated in subjects with PE (5.7 [4.5;6.5] μg/l) as compared to healthy, age-matched, pregnant controls (4.6 [4.1;5.6] μg/l) ($p<0.05$) after adjusting for BMI and gestational age at blood sampling (Table 1). Systolic (SBP) and diastolic (DBP) blood pressure, gestational age at blood sampling, creatinine, FFA, TG, CRP, leptin, and adiponectin were significantly elevated in patients with PE as compared to controls ($p<0.05$) (Table 1). In contrast, gestational age at delivery, birth weight, and GFR were significantly decreased in PE patients as compared to control subjects ($p<0.05$) (Table 1). In addition, BMI, measures of insulin sensitivity (FI and HOMA-IR), as well as total and HDL cholesterol, were not different between the two groups (Table 1).

Table 1
Baseline characteristics of the study population during pregnancy. BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; FFA, free fatty acids; FG, fasting glucose; FI, fasting insulin; Gas6, growth arrest specific protein 6; GFR, glomerular filtration rate; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein; PE, preeclampsia; SBP, systolic blood pressure; TG, triglycerides. Values for median (interquartile range) are shown.

	Control	PE
N	51	51
Gas6 (μg/l)	4.6 (4.1;5.6)	5.7 (4.5;6.5)*†
Age (years)	30 (27;34)	30 (24;35)
BMI (kg/m^2)	22.0 (20.3;24.6)	22.7 (21.5;27.3)
SBP (mm Hg)	115 (105;124)	159 (146;173)*†
DBP (mm Hg)	70 (62;76)	100 (90;108)*†
Gestational age at blood sampling (completed weeks)	28 (27;30)	29 (27;33)#
Gestational age at delivery (completed weeks)	39 (35;40)	30 (28;33)*†
Birth weight (g)	3115 (2310;3525)	1215 (879;1783)*†
Creatinine (μmol/l)	54 (48;58)	66 (60;76)*†
GFR (ml/min)	121.8 (116.7;126.8)	105.1 (92.9;117.8)*†
FG (mmol/l)	3.6 (3.2;4.1)	3.8 (3.3;4.8)*
FI (pmol/l)	58.9 (38.5;76.8)	73.9 (46.7;111.1)
HOMA-IR	1.3 (0.8;1.8)	1.7 (1.0;3.2)
FFA (mmol/l)	0.4 (0.3;0.6)	0.8 (0.6;1.1)*†
Cholesterol (mmol/l)	6.5 (5.9;7.6)	6.7 (6.0;7.8)
HDL cholesterol (mmol/l)	1.8 (1.5;2.2)	1.8 (1.4;2.1)
LDL cholesterol (mmol/l)	3.9 (3.4;4.7)	3.4 (2.8;4.1)*
TG (mmol/l)	2.2 (1.7;2.8)	3.4 (2.9;4.0)*†
CRP (mg/l)	2.5 (1.4;5.2)	8.2 (3.4;18.3)*†
Leptin (μg/l)	20.4 (14.4;26.2)	41.0 (28.5;65.4)*†
Adiponectin (mg/l)	7.4 (5.2;9.2)	11.7 (7.5;17.5)*†

* Indicates $p<0.05$ as compared to control as assessed by Mann–Whitney-*U* test after adjustment for BMI and gestational age at blood sampling.

† Indicates $p<0.05$ as compared to control as assessed by unpaired Student's *t*-test after adjustment for BMI and gestational age at blood sampling with Bonferroni adjustment for multiple testing.

\# Indicates $p<0.05$ as compared to control as assessed by Mann–Whitney-*U* test.