

Increased serum ghrelin in preeclampsia: Is ghrelin a friend or a foe?

Podwyższony poziom greliny w stanie przedrzucawkowym:
czy grelina jest przyjacielem czy wrogiem?

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Abstract

Objectives: To investigate maternal serum ghrelin levels in pregnancies complicated by preeclampsia and to explore the relationship between ghrelin level and disease severity.

Materials and methods: This case-control study included 40 healthy pregnant women, 42 women with mild preeclampsia, and 40 women with severe preeclampsia. The groups were matched in terms of maternal and gestational age and body mass index. Serum ghrelin levels were measured via enzyme immunoassay.

Results: Serum ghrelin levels were significantly higher in women with mild and severe preeclampsia than in healthy controls ($p < 0.001$). Although serum ghrelin levels were somewhat higher in the severe compared to the mild preeclampsia group, the difference was not statistically significant ($p > 0.05$). In the control group, no significant correlation was observed between ghrelin level and any other parameter, but in the preeclampsia group, serum ghrelin levels were negatively correlated with uterine artery Doppler index values and both systolic and diastolic blood pressure (all p -values < 0.05). Multivariate stepwise linear regression analysis revealed that systolic blood pressure ($\beta = 0.493$, $p = 0.023$) was independently associated with serum ghrelin level.

Conclusion: Elevated blood ghrelin levels were correlated with disease severity in pregnancies complicated by preeclampsia.

Key words: **ghrelin / pregnancy / preeclampsia /**

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Streszczenie

Cel pracy: Ocena poziomu greliny w surowicy kobiet w ciąży powikłanej stanem przedzucawkowym i określenie związku między poziomem greliny a ciężkością choroby.

Materiał i metoda: Do badania włączono 40 zdrowych kobiet w ciąży, 42 z łagodnym stanem przedzucawkowym i 40 z ciężkim stanem przedzucawkowym. Grupy były dobrane pod względem wieku ciążowego, wieku matek i wskaźnika masy ciała. Poziom greliny w surowicy był mierzony metodą immunoenzymatyczną.

Wyniki: Poziom greliny w surowicy był istotnie wyższy u kobiet z łagodnym i ciężkim stanem przedzucawkowym niż w grupie kontrolnej ($p < 0.001$). Chociaż poziom greliny w surowicy był wyższy w grupie z ciężkim stanem przedzucawkowym, to ta różnica nie była istotna statystycznie ($p > 0.05$). W grupie kontrolnej nie obserwowano żadnych istotnych związków pomiędzy poziomem greliny a jakimkolwiek innym parametrem, ale w grupie ze stanem przedzucawkowym poziom greliny w surowicy był ujemnie skorelowany z indeksami przepływu Dopplera w tętnicy macicznej oraz ciśnieniem krwi skurczowym i rozkurczowym (all p -values < 0.05). Wieloczynnikowa analiza regresji liniowej wykazała, że skurczowe ciśnienie krwi było niezależnym czynnikiem związanym z poziomem greliny w surowicy ($\beta = 0.493$, $p = 0.023$).

Wnioski: Podwyższony poziom greliny we krwi był związany z ciężkością choroby w ciążach powikłanych stanem przedzucawkowym.

Słowa kluczowe: **grelina / ciąża / stan przedzucawkowy /**

Introduction

Preeclampsia is a serious complication of pregnancy and is clinically characterized by maternal hypertension and proteinuria after 20 weeks of gestation; the condition affects 3–5% of all pregnancies and is associated with substantial maternal and neonatal morbidity and mortality [1]. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia remain poorly understood. Increasing evidence suggests that an inadequate maternal vascular response to placentation, vascular endothelial dysfunction, abnormal angiogenesis, and an exaggerated inflammatory response featuring systemic oxidative stress may play important roles in the pathogenesis of this pregnancy-specific disorder [2].

Adipose tissue is now generally considered not simply a passive energy store but also an active endocrine organ influencing many metabolic activities. Adipocytes produce and secrete several mediators collectively termed adipokines. Some of these, including leptin, adiponectin, visfatin, resistin, and ghrelin, play important roles in regulating energy homeostasis, insulin sensitivity, lipid/carbohydrate metabolism, and inflammatory processes. Also, adipokines regulate maternal energy metabolism and insulin sensitivity during gestation, and they have been implicated in the development of various complications of pregnancy, including gestational diabetes mellitus, fetal growth restriction, and preeclampsia [3].

Ghrelin is a 28-amino acid peptide hormone produced predominantly by the stomach. It is a potent stimulator of growth hormone secretion and acts by binding to the growth hormone secretagogue receptor [4]. Ghrelin and its receptor are expressed at low levels in the central nervous system, adipose tissue, endocrine organs, muscle tissue, and the gastrointestinal tract where ghrelin exerts profound physiological activities [5]. Ghrelin in the systemic circulation exists in two major isoforms: acylated (active) and des-acylated (inactive). Ghrelin levels are enhanced by food deprivation and decrease after food intake. Ghrelin acts both centrally and peripherally, and plays significant roles in regulation of energy metabolism. Moreover, ghrelin controls gastrointestinal motility, pancreatic secretion, and

cell proliferation and exerts antiinflammatory and antioxidant effects [6]. Ghrelin has been shown to lower peripheral vascular resistance, either directly at the vascular level or by centrally modulating sympathetic nervous system activity [7]. Furthermore, ghrelin administration improves endothelial dysfunction [8].

The objective of the current study was to investigate ghrelin levels in normal pregnancies and those complicated by preeclampsia. We speculated that the circulating ghrelin concentration might reflect disease severity.

Materials and methods

This case-control study was conducted at the Antalya Training and Research Hospital, Antalya, Turkey, between March 2013 and January 2014. Eligible participants were recruited from the delivery service of our institution. The Ethics Committee of the institution approved the study, and all patients provided written informed consent.

The study group consisted of 42 women with mild preeclampsia and 40 with severe preeclampsia. All subjects had late-onset preeclampsia, diagnosed at gestational week 34 or later. The control group consisted of 40 normotensive healthy pregnant women. The groups were matched in terms of maternal and gestational age and body mass index (BMI). All subjects were non-smokers and had similar demographic backgrounds, and all were admitted for delivery. Additionally, we selected only women who delivered via elective cesarean section, thus eliminating any possible influence of labor or premature membrane rupture. The indication for elective cesarean section in all patients was a prior cesarean section. Patients with multiple fetuses, chronic hypertension, HELLP syndrome, diabetes mellitus, prior renal or hepatic function, or a vascular or inflammatory disease were excluded.

Preeclampsia was diagnosed as the presence of hypertension (systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg on at least two occasions 6 h apart) and proteinuria (≥ 300 mg in a 24-h urine collection or a score of ≥ 1 on at least one dipstick measurement) after week 20 of gestation as defined by the International Society of Hypertension in Pregnancy [9].

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Blood pressure was measured in the sitting position after 10 min of rest. Preeclampsia was classified as severe if a woman had one or more of the following symptoms: blood pressure of ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic, $\geq 3+$ protein by dipstick test in two urine samples taken 4 h or more apart or 5 g of protein in 24-h urine sample, epigastric or right upper-quadrant pain, blurred vision, cerebral disturbance, abnormal liver function, pulmonary edema or cyanosis, low platelet level; and oliguria (less than 500 mL urine in 24 h). Gestational age was determined by the last menstrual period and confirmed via ultrasonographic examination performed during the first trimester. BMI was calculated as weight (kg)/height (m^2).

All patients underwent Doppler examination at admission prior to delivery. All examinations were performed using a 2–7 MHz transabdominal transducer (Mindray DC-T6; Shenzhen, China) with patients in the lateral decubitus position to avoid supine hypotension. Doppler measurements were performed in the absence of fetal movement and upon voluntary suspension of maternal breathing. Spectral Doppler parameters were determined automatically from three or more consecutive waveforms, with the angle of insonation being as close to 0° as possible. Umbilical artery Doppler velocimetry was performed on a free loop of the umbilical cord remote from the sites of fetal and placental insertion. Both uterine arteries were assessed at the place where they crossed the external iliac arteries. The mean value of both uterine arteries was calculated and used in statistical analysis.

None of the preeclamptic patients or controls received any medication prior to blood sampling. A fasting venous blood sample (5 mL) was obtained from each subject prior to cesarean section. Samples were centrifuged at 3000 rpm for 15 min at 4°C to separate serum, which was aliquoted and stored at -80°C prior to assay. Ghrelin levels were measured using a commercially available ELISA kit (USCN Life Science, Inc., Houston, TX, USA; Catalog # CEA991Hu) according to the manufacturer's instruction. The assay sensitivity was 0.6 pg/mL. The intra- and inter-assay coefficients of variation were $<10\%$ and $<12\%$, respectively. All absorbances were measured on an ELX 800 ELISA reader (BioTek Instruments, Inc., Winooski, VT, USA). The assay results are expressed as pg/mL.

Statistical analysis

Data distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables are presented as mean \pm standard deviation if normally distributed or as median (minimum–maximum) if not. Comparison of numerical variables between groups were performed using the Kruskal–Wallis test, and post hoc comparisons were made using the Mann–Whitney U-test with Bonferroni's correction if the data were not normally distributed. Correlations between ghrelin levels and other variables were evaluated using Spearman's rank test. Multivariate stepwise linear regression analysis was performed to identify independent relationships between the circulating ghrelin level and other variables. Those variables that showed significant correlations were included as independent variables in the regression model. All tests were two sided, and the significance level was set at $p < 0.05$. Statistical analysis was performed using the SPSS statistical package for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics, Doppler parameters, and neonatal outcomes of the study groups are shown in Table I. As participants were matched in terms of maternal and gestational age and BMI, these parameters were similar among all groups ($p > 0.05$). As expected, systolic and diastolic blood pressure and the Doppler indices of the umbilical and uterine arteries were significantly higher in the severe preeclampsia group than in the control and mild preeclampsia groups ($p < 0.05$), and birth weight was significantly lower ($p < 0.05$).

There was a statistically significant between-group difference in serum ghrelin levels ($p < 0.001$): 45.83 ± 12.28 pg/mL in the control group, 55.54 ± 2.16 pg/mL in the mild preeclampsia group, and 58.69 ± 6.02 pg/mL in the severe preeclampsia group (Figure 1). Upon Bonferroni testing, the serum ghrelin levels were significantly higher in the mild and severe preeclampsia groups than that in the control group ($p < 0.001$). However, no significant difference was evident between the mild and severe preeclampsia groups ($p = 0.06$).

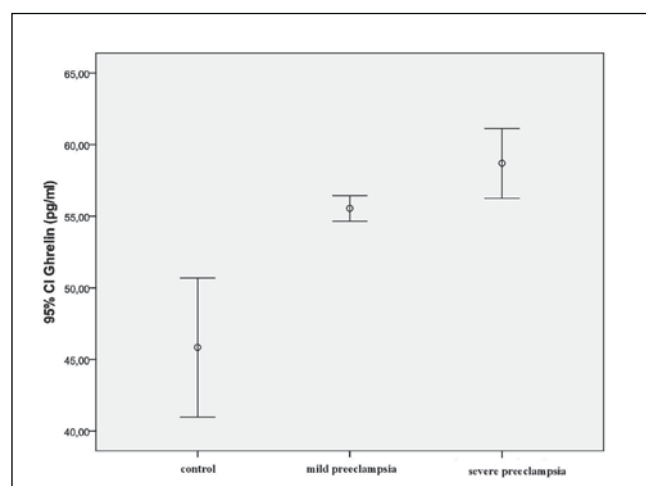


Figure 1. Distribution of serum ghrelin levels in the control, mild and severe preeclampsia groups.

The relationships between serum ghrelin level and other variables are shown in Table 2. No significant correlation was observed between serum ghrelin level and any other variable in the control group. However, serum ghrelin levels were negatively correlated with both systolic and diastolic blood pressure ($r = -0.591$, $r = -0.541$, respectively; $p < 0.001$ for both) and uterine artery pulsatility index ($r = -0.334$, $p = 0.003$), and resistance index ($r = -0.362$, $p = 0.015$) in the preeclampsia group. Multivariate stepwise linear regression analysis revealed that systolic blood pressure ($\beta = 0.493$, $p = 0.023$) was an independent factor influencing serum ghrelin levels.

Discussion

We found that serum ghrelin levels were significantly higher in patients with preeclampsia than healthy controls. Although the levels tended to be higher in patients with severe compared to mild preeclampsia, this difference was not statistically significant. Moreover, ghrelin levels were negatively correlated with both

Table I. Comparison of clinical characteristics and Doppler parameters of the study groups.

Variables	Control (n=40)	Mild preeclampsia (n=42)	Severe preeclampsia (n=40)	p	p ¹	p ²	p ³
Age	28.1±3.7	27.7±4.8	28.2±2.8	0.284			
Gestational age (week)	38.9±0.5	38.6±2.2	38.5±2.5	0.245			
BMI (kg/m ²)	30.6±1.1	30.8±2.2	30.7±2.4	0.467			
Gravidity	3 (1-5)	2 (1-4)	2 (1-5)	0.164			
Systolic blood pressure (mmHg)	100.1±7.4	147.9±6.4	166.4±8.2	<0.001	<0.001	<0.001	<0.001
Diastolic blood pressure (mmHg)	70±7.4	96.6±5.5	110.2±7.9	<0.001	<0.001	<0.001	<0.001
Umbilical artery PI	0.65±0.12	0.70±0.14	1.28±0.23	<0.001	0.445	<0.001	<0.001
Umbilical artery RI	0.41±0.11	0.47±0.15	0.70±0.18	<0.001	0.255	<0.001	0.002
Uterine artery PI	0.59±0.19	0.63±0.18	1.25±0.11	0.001	0.131	<0.001	<0.001
Uterine artery RI	0.36±0.14	0.42±0.11	0.67±0.12	0.01	0.335	0.001	<0.001
Birth weight (g)	3548±232	3307±410	2768±312	<0.001	0.035	<0.001	<0.001

Values are given as mean ± SD (standard deviation) or median (range).
p – between three groups; p¹ – between mild preeclampsia and control; p² – between severe preeclampsia and controls;
p³ – between severe preeclampsia and mild preeclampsia.
BMI – body mass index, PI – pulsatility index, RI – resistance index.

Table II. Correlations between serum ghrelin levels and other variables assessed in the control and preeclampsia groups.

	Control group		Preeclampsia group	
	r	p	r	p
Gestational age	0.183	0.362	0.025	0.862
BMI	0.37	0.058	0.11	0.441
Systolic blood pressure	-0.113	0.576	-0.591	<0.001*
Diastolic blood pressure	-0.177	0.377	-0.541	<0.001*
Umbilical artery PI	-0.143	0.478	0.187	0.105
Umbilical artery RI	-0.217	0.277	0.118	0.302
Uterine artery PI	-0.093	0.645	-0.334	0.003*
Uterine artery RI	-0.143	0.476	-0.362	0.015*
Birth weight	0.152	0.448	0.039	0.27

BMI – body mass index, PI – pulsatility index, RI – resistance index, * Significant difference

systolic and diastolic blood pressure, and the values of uterine artery indices that are associated with preeclampsia severity.

Ghrelin is now considered to play pivotal roles in both normal and complicated pregnancies. Ghrelin and its functional receptor are expressed in the placenta. Ghrelin levels begin to increase in the first trimester, peak at mid-pregnancy, and fall to their lowest levels in the third trimester [10]. The physiological actions of ghrelin during pregnancy are thought to reflect an interaction of ghrelin with human placental growth hormone, altering maternal metabolism to favor development of maternal insulin resistance, in turn increasing fetal nutrient availability [11].

Additionally, ghrelin stimulates fetal growth by enhancing cell proliferation [12]. In support of such a role for ghrelin, umbilical cord ghrelin levels were shown to be higher in small-

for-gestational-age neonates compared with appropriate- and large-for-gestational-age neonates [13]. Furthermore, elevated ghrelin levels were observed in patients with hypertensive disorders of pregnancy, which are risk factors for fetal growth restriction [14, 15]. We corroborated this finding; an important observation of the present study was that the increased ghrelin levels in preeclampsia appeared to be correlated with the severity of the disorder.

Recent evidence has indicated that ghrelin exerts beneficial cardioprotective effects and may be useful to treat heart failure and hypertension [16]. Administration of ghrelin to healthy individuals causes vasodilation, decreases blood pressure and cardiac after-load, and increases cardiac output, without affecting heart rate [17]. Low plasma ghrelin levels were associated with

hypertension and were inversely correlated with blood pressure elevation [18]. In contrast, we observed that increased ghrelin levels in preeclampsia were negatively correlated with both systolic and diastolic blood pressure. Despite the positive effects exerted by ghrelin on the cardiovascular system, the mechanisms triggering increased ghrelin levels in preeclampsia remain unclear. Three potential mechanisms, compensatory, inhibitory, and angiogenetic, may be in play; we discuss these below.

Despite extensive research, the detailed pathophysiology of preeclampsia remains poorly understood. It has been suggested that fetal hypoperfusion and increased placental vasoconstrictive mediators caused by inappropriate placentation eventually trigger maternal hypertension [19]. Appropriate placentation and fertilization play critical roles in the early stages of pregnancy. In turn, these processes are greatly affected by systemic and local actions of mediators including pregnancy-associated placental protein A, insulin-like growth factor 1, vascular endothelial growth factor (VEGF), and nitric oxide (NO) [20-22]. These materials lower blood pressure during the early stages of pregnancy. Alterations in the levels of these bioactive substances and/or increases in the antagonistic effects of anti-VEGF (anti-soluble fms-like tyrosine kinase) or anti-NO (soluble endoglin) factors may trigger maternal hypertension [23, 24]. We speculate that the increased ghrelin levels in women with preeclampsia may indicate that a compensatory physiological mechanism has been triggered in response to impairment of the physiological balance among various important mediators.

A second mechanism of ghrelin action may be inhibitory in nature. During embryogenesis, extravillous trophoblasts attain the uterine decidual spiral arterioles and organize the vessels to supply high volumes of low-pressure blood to the fetus [25]. All of hemodynamic factors, regulatory enzymes, cytokines, immune cells, and (several) hormones play significant roles in such vascular remodeling. Previous studies found that ghrelin regulated the secretion of human chorionic gonadotropin, VEGF, progesterone, estrogen, follicle-stimulating hormone, and lutenizing hormone [26-29]. Additionally, ghrelin interferes with embryo implantation in an immunomodulatory manner [27]. Changes in ghrelin levels during the early stages of pregnancy may impair both immunity and the functions of mediators essential for placental vascular remodeling. Any abnormality in this process causes inappropriate placentation, leading to development of preeclampsia.

Any angiogenetic effect of ghrelin is rather speculative. Zaniolo et al. showed that ghrelin and its receptor were expressed in retinal endothelial cells. Ghrelin promoted pathological angiogenesis during retinal neovascularization, which was significantly inhibited by ghrelin receptor antagonists [30]. Increased ghrelin levels may stimulate a similar pathophysiological mechanism during remodeling of the decidual arterioles, triggering a cascade of pathophysiological events leading to development of preeclampsia.

Conclusions

Serum ghrelin levels were markedly higher in women with preeclampsia and were associated with the severity of the condition. Although various potential mechanisms of action have been suggested, it remains unclear whether ghrelin levels rise in the initial or later stages of preeclampsia. Reliable biochemical

markers for prediction and diagnosis of preeclampsia would have a great impact on maternal health and several have been suggested. It can reduce unnecessary suffering and health care costs by early detection of mothers at increased risk for preeclampsia, thus avoiding unnecessary hospitalization of pregnant women with suspect or mild preeclampsia and enabling monitoring of the progression of the disease thereby, optimizing time for delivery and hopefully reducing the number of premature births. However, further studies are needed to clarify the significance and the underlying mechanisms of the increased ghrelin levels in preeclampsia and prospective clinical applying of ghrelin in clinical practice.

Authors' contribution:

1. Onur Erol – study design, analysis, assumptions, interpretation of data, corresponding author.
2. Hamit Y. Ellidağ – study design, acquisition of data.
3. Hülya Ayık – concept, assumptions, study design.
4. Gül A. Bülbül – acquisition of data.
5. Aysel U. Derbent – revised article critically.
6. Sibel Kulaksızoğlu – acquisition of data.
7. Necat Yılmaz – revised article critically.

Authors' statement

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