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Circulating ADAMTS-12 levels in early- and late-onset severe preeclampsia

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Abstract

Aim of this study is to determine whether maternal serum ADAMTS-12 levels differ among early-onset (<34th week) severe preeclampsia (EOS-PE), late-onset (≥ 34th week) severe preeclampsia (LOS-PE), and uncomplicated pregnancies. In this case-control study, maternal serum samples obtained from 22 pregnant patients with EOS-PE, 25 pregnant patients with LOS-PE, and 35 healthy patients with uncomplicated pregnancies (control group). The distinctly higher rate of negative perinatal outcomes in both EOS-PE and LOS-PE patients were recorded. There were no significant differences among the ADAMTS-12 levels of the groups. The main questions that need to be answered are whether the only difference between these two diseases is the time of their onset and whether the only difference between them with respect to fetal morbidity and mortality is prematurity.

Keywords: Preeclampsia, ADAMTS-12, placenta, trophoblast, matrix metalloproteinase

Introduction

Preeclampsia (PE), a pregnancy-specific syndrome is characterized by de-novo hypertension and proteinuria after 20th weeks of gestation [1]. The etiology of the disease is heterogeneous and has not yet been identified. Multiple factors have been suggested in its pathophysiology, such as inflammation, defective placentation; cardiovascular maladaptation to pregnancy; malfunction in immunological, hormonal, genetic, and angiogenic mechanisms and the increased oxidative stress [1-4].

According clinical severity and laboratory findings of the disease, PE is classically divided into mild and severe forms. Especially severe form of PE carries the high risks of disseminated intravascular coagulation; stroke, liver infarction and abruption of placentae, pulmonary edema, renal failure and thereby, may be cause of fetal and maternal morbidity and mortality [1,2].

PE can be classified on the timing of disease onset, as early-onset (EO), occurring before 34th gestational week and late-onset (LO) occurring 34th gestational week or later. Despite the presenting characteristic overlap, EOS- and LOS-PE are associated with different clinical features, biochemical markers and maternal and fetal results. It is suggested that while EO- PE is primarily associated with defective placentation; LO-PE likely occurs due to a failure of maternal adaptation to pregnancy [3].

Endovascular invasion of the spiral arteries by extravillous trophoblast (EVT) is a crucial step for both initial implantation and subsequent placentation [1,2,5].

Endovascular trophoblasts invade and transform spiral arteries during early pregnancy. Upregulation of proteolytic enzymes and matrix metalloproteinases (MMPs) is accompanied to the invasion process. Altered placental expression of MMPs has been associated with shallow invasion of EVT and incomplete remodeling of the spiral arteries resulting PE [6].

A Disintegrin-like Metalloproteinase with Thrombo Spondin motif (ADAMTS) constitute a family of secreted endopeptidases related to MMPs. ADAMTS family were known to play important roles in numerous biological and pathological processes, such as angiogenesis and embryonic development, inflammation, atherosclerosis and malignancy [7]. Although multiple ADAMTS subtypes showed in human placenta, it has been demonstrated that ADAMTS-12 were preferably expressed by invasive EVT [8].

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Previously the low maternal serum ADAMTS-12 has been associated with PE [9]. However, it is not known about the relationship between ADAMTS-12 and its role in *early*- versus *late-onset severe PE*. For this reason we aimed to evaluate, first time in the literature, to determine whether serum ADAMTS-12 differ in early onset severe PE (EOS-PE) and late-onset severe PE (LOS-PE), and uncomplicated pregnancies or not.

Materials and methods

This case control study was conducted between August 2015 and January 2016 at the Zekai Tahir Burak Women Health Education and Research Hospital, which is a tertiary referral hospital in Ankara, Turkey. Consecutive singleton pregnancies between the ages of 18 and 35 years and met all of inclusion criteria were included in the study. The Institutional Review Board approved the study. Informed consent was obtained from all of the participants.

PE was defined as new onset hypertension (≥140/90 mmHg) and proteinuria. Proteinuria was defined as excretion of at least 300 mg protein in a 24-hour urine sample. The diagnosis of severe PE was based on the presence of any of the following criteria: systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mmHg on two separate measurements performed at six-hour intervals at the least, increased serum creatinine (>1.1 mg/ dL), headache, visual impairment, epigastric or right upper quadrant pain, elevated hepatic enzymes, thrombocytopenia (PLT<100000/mm^3), or pulmonary edema [1]. Pregnant with severe PE were divided into two groups: early-onset severe PE (<34^{th} week) (EOS-PE) and late-onset severe PE (≥34 weeks) (LOS-PE) [2]. Fetal growth restriction (FGR) was diagnosed when estimated fetal weight was below the 10th percentile, with abnormal fetal Doppler parameters. Perinatal mortality was defined the sum of fetal deaths plus early neonatal deaths (deaths within the first seven days of birth [10]. The first day of the last menstrual period and/or first trimester ultrasonography measurement of the crown–rump length was used for gestational age was determination. A Voluson 730 Expert scanner (GE Medical systems, Kretztechnik GmbH & OHG, Zipf, Austria) was used for ultrasonography and Doppler evaluations.

Women with multiple gestation, any systemic disease including cardiovascular, endocrinological, metabolic, inflammatory and autoimmune disorders, pre-pregnancy obesity (BMI ≥30 kg/m^2), and who smoke or on any drug other than iron medication and women who complicated with HELLP syndrome were excluded from the study. All PE cases were hospitalized and followed up with a standard institutional protocol compatible with the one defined previously [11]. The maternal age, body mass index (BMI), resting blood pressure, and results of the complete blood count and biochemical tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid dehydrogenase (LDH), total bilirubin, creatinine and blood urea nitrogen (BUN), of each participant were recorded.

Gestational age at delivery, mean birth weight, birth weight <10^{th} percentile, 5 minute Apgar score, neonatal intensive care unit (NICU) admission and perinatal mortality were also recorded as perinatal outcome parameters.

All blood samples were collected at first admission by venipuncture prior to delivery or administration of betamethasone to accelerate fetal lung maturation or any other drugs (e.g. magnesium sulphate (MGSO4). The serum samples were centrifuged at 5000 revolutions/min for 10 minutes within 5-10 minutes of blood sampling and frozen immediately at −80°C until the analyses were performed.

Biochemical Measurements:

The rest of the blood analyses other than ADAMTS-12, were carried out within two hours of blood sampling, using a hematology analyzer (GEN-S; Beckman-Coulter Inc., Brea, CA) at the central laboratories of our hospital. Levels of ADAMTS-12 were measured with commercially available human ADAMTS-12 ELISA Kit (CK-E91160 Eastbiopharm, Hangzhou Eastbiopharm Ltd, China). All samples were assessed in duplicate. The measurement ranges for ADAMTS-12 was 2–100 ng/mL. The intra and inter assay coefficients of variation for ADAMTS-12 were 6% and 8%, respectively.

Statistical Analysis

The statistical analyses were performed using the Statistical Package for the Social Sciences version 15.0 (SPSS, Chicago, IL, USA). Sample size was determined according to the results of the central limit theorem. The Shapiro-Wilk test for used to examine the continuous variables with normal and abnormal distributions. The one-way analysis of variance (One-Way ANOVA) test was used for the normally distributed continuous variables, and the Kruskal-Wallis Test was used for the abnormally distributed continuous variables. When the Kruskal-Wallis test indicated significant differences, the causes of those differences were determined using a Bonferroni-adjusted Mann-Whitney U test. Nominal variables were analyzed by Pearson's x^2 or Fisher's Exact Test when applicable. Continuous variables are presented as means ± SD and categorical variables are presented as number of cases (%). The optimal cutoff points of laboratory parameters determining PE was evaluated Receiver operating characteristic (ROC) analysis. Correlations between different maternal biochemical parameters were examined using Spearman's correlation. P values <0.05 were considered statistically significant.

Results

The study groups consisted of hospitalized, eventually delivered and who met inclusion criteria, 22 pregnant with a diagnosis of EOS-PE and 25 pregnant with diagnosis of LOS-PE. As the control group maternal age, gravidity and BMI matched 35 healthy consecutive pregnant women were recruited within the same time interval.

The demographic and of the groups are shown in Table 1. As expected both PE groups' blood pressure levels were higher than the control group. But, there was no significant difference between EOS-PE and LO-PE groups in term of systolic and diastolic blood pressure. Gestational age at serum sampling was significantly lower in the EOS- PE compared to the LOS-PE group.

Laboratory characteristics of the groups were presented in Table 2. Serum ADAMTS-12 levels were comparable among all groups.

Table 1. The comparison of patient characteristics in the control (group 1), EOS-PE (group 2) and LOS-PE (group 3) groups.

	Group 1 (n= 35)	Group 2 (n= 22)	Group 3 (n= 25)	p value (groups)		
				1 vs 2	1 vs 3	2 vs 3
Maternal age (year)	27.5± 6.2	26.2± 7.1	25.9± 7.3	NS	NS	NS
BMI (kg/m^2)	25.3± 3.7	26.9± 4.1	26.3± 3.9	NS	NS	NS
Gravidity (range)	2(0-2)	2(1-3)	3(1-3)	NS	NS	NS
Nulliparity, n (%)	15 (42%)	10 (45%)	11 (44%)	NS	NS	NS
GA at assessment, week (range)	34 (33-37)	32 (27-33)	36 (34-39)	NS	NS	0.01
Systolic BP (mmHg)	119.6± 10	169.7± 8	171.5± 12	<0.001	<0.001	NS
Diastolic BP (mmHg)	82± 9	109.4± 10	110.3± 13	<0.001	<0.001	NS

Data expressed as mean ± SD, * The mean difference is significant at the 0.05 level. NS: Non-significant.
BMI: body mass index, GA: Gestational age, BP: Blood pressure.

Table 2. The comparison of hematological and biochemical results of the control (group 1), EOS-PE (group 2) and LOS-PE (group 3) groups.

	Group 1 (n = 35)	Group 2 (n=22)	Group 3 (n = 25)	p value (groups)		
				1 vs 2	1 vs 3	2 vs 3
Hemoglobin, g/dL	11.9± 0.6	13.9± 2.1	13.7± 1.1	<0.001	<0.001	NS
Hematocrit, %	35.8± 3.6	39.4 ±4.0	40.2± 4.5	<0.001	<0.001	NS
WBC (10^3/ µL)	8.9 ±4.1	9.8± 2.4	9.1 ±2.3	NS	NS	NS
Platelet, x10^3/mm^3	226± 26	125± 67	121± 39	<0.001	<0.001	NS
BUN, mg/dL	13± 11.4	24.3± 16.1	25.3± 10.5	<0.001	<0.001	NS
Creatinine, mg/dL	0.53± 0.2	0.71± 0.4	0.69± 0.1	<0.001	0.03	NS
LDH, U/L	285± 102	618± 304	593± 278	<0.001	<0.001	NS
AST, U/L	7.3± 6	42.3± 22.1	48.6± 10.8	<0.001	<0.001	NS
ALT, U/L	9.3± 7.2	35.2± 79.9	39.4±16.3	<0.001	<0.001	NS
ADAM-TS12, ng/ml	40.06± 37.5	27.30± 23.6	32.2± 30	NS	NS	NS

Data expressed as mean ± SD, *the mean difference is significant at the 0.05 level. NS: Non-significant.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, BUN; Blood urea nitrogen, LDH: Lactate dehydrogenase, WBC: white blood cell

The perinatal outcomes of the groups were presented in Table 3. In the EOS-PE group 13 of the 22 women (59%) were determined to have FGR. In this group all 22 pregnant women were delivered by cesarean section (C/S) except the 4 of them (C/S rate was 81%). We have two fetal demises and 6 early neonatal deaths (within the first seven days of birth) in the EOS-PE group with perinatal mortality rate of 36%. There were no cases of fetal demise in LOS-PE and the control groups. Three early neonatal deaths were noted in the LOS-PE group. The main indications for admission to the NICU were neonate respiratory problems and preterm delivery. There was no maternal mortality in the groups.

Table 3: The perinatal outcomes of the control (group 1), EOS-PE (group 2), LOS-PE (group 3) groups.

	Group 1 (n= 35)	Group 2 (n= 22)	Group 3 (n = 25)	p value (groups)		
				1 vs 2	1 vs 3	2 vs 3
Delivery week	40.1± 3.1	32.6± 1.8	36.4± 2.7	<0.001	<0.001	<0.001
Delivery Mode- C/S, n (%)	11(31.4%)	18 (81%)	15 (60%)	<0.001	<0.001	<0.001
Preterm Delivery	-	22 (100%)	14 (56%)	-	-	<0.001
Mean BW, gram	3481± 600	1220± 890	2581± 650	<0.001	<0.001	<0.001
5 minute Apgar score	9 (8-10)	6 (3-8)	8 (5-10)	<0.001	<0.001	<0.001
NICU admission, n(%)	-	20 (90%)	9 (36%)	<0.001	<0.001	<0.001
FGR, n(%)	-	13 (59%)	9 (36%)	<0.001	<0.001	0.05
Fetal Demise	-	2 (9%)	-	<0.001	<0.001	<0.001
Perinatal mortality*, n(%)	-	8 (36%)	3(2%)	<0.001	<0.001	<0.001

Data expressed as number (%), mean ± SD. *The mean difference is significant at the 0.05 level; NS: Non-significant.
BW; Birth weight, C/S; caserean section, FGR; Fetal growth restriction, NICU; Neonatal intensive care unit.
*Perinatal mortality was defined the sum of fetal deaths plus early neonatal deaths (deaths within the first seven days of birth).

Discussion

We designed this study due to the discussions in the literature stating that EOS-PE and LOS-PE are two different diseases with different etiopathogeneses. In order to shed light on the pathogenesis of EOS-PE and LOS-PE we investigated, first time in the literature, the maternal serum ADAMTS-12 levels in a group of women with severe PE and compared the results with a healthy pregnant group. Additionally we also presented perinatal outcomes of pregnant with EOS-PE and LOS-PE. In this study, no significant difference was detected in ADAMTS-12 levels among the groups.

Placental development that essential for maintaining pregnancy is tightly regulated by a large number of molecules such as growth factors and growth factor binding proteins, pro- inflammatory cytokines, and proteoglycans and MMPs, integrins, cadherins and proteases that regulate cell–cell or cell–matrix adhesion and extracellular matrix (ECM) degradation. Remodeling of spiral arterioles that increases the blood flow towards the placenta and fetus requires signals from both fetal and maternal uterine compartments [5]. The regulation of MMP activity seems to be critical in these steps [5,12]. Presence of a dysregulation in the tightly controlled process of placentation is posited to further cause of pregnancy complications such as PE, FGR, preterm delivery and/ or placental abruption [5]. Failed trophoblast invasion is accepted to be a central pathogenetic mechanism in PE. It has been suggested that PE is a two-stage disease; first, the defective placentation, and second, the maternal systemic syndrome [1,5]. However, the definite mechanism that underlies incomplete invasion of EVT in the PE remains unknown.

Previous studies suggested that all members of ADAMTS family have the potential to act as metalloproteinase and to regulate cell adhesion [7]. In a well-designated study, many members of ADAMTS family including -1, -2, -4, -5 -6, -7, -9, and -12 mRNA levels were detected in first trimester human placenta. The investigators observed that high ADAMTS-12 expression invasive human trophoblastic cells. They suggested that ADAMTS-12 regulate trophoblast differentiation along the invasive pathway via a catalytic independent mechanism that necessitated integrin-ECM mediated interaction. They discussed that ADAMTS-12 may play an important role for placental adhesion to extracellular matrix; it increases of invasion of the EVT [8]. In view of these observation, we examined the ADAMTS-12 levels in the groups. Therefore lower levels of ADAMTS-12 may be a marker of EVT invasion in PE. But no significant difference was detected in ADAMTS-12 levels among the groups. If sample size of the groups were larger, the levels of ADAMTS-12 might be found statistically significant.

We acknowledge the limitations that the small sample size partly due to the fact that we had to exclude women who had already on Mgso4 or steroid treatment for fetal lung maturity at the time of admission to our center or who had comorbid diseases. One of the strength of our study is the homogeneity of the characteristics of the women in the study groups and our prospective study design. The patients had either with and without PE were followed until delivery. Despite our best efforts to adjust for clinically relevant characteristics, the possibility of residual confounding remains.

In conclusion, herein, first time in the literature, we demonstrated that unchanged circulating levels of ADAMTS-12 in EOS- and LOS-PE. The main questions that need to be answered are whether the only difference between these two diseases is the time of their onset and whether the only difference between them with respect to fetal morbidity and mortality is prematurity. However, the precise underlying mechanism is still unclear and needs further studies for better understanding of PE pathogenesis.

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