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ELABELA Plasma Concentrations are Increased in Women with Late-Onset Preeclampsia

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Abstract

Objective: ELABELA is a newly discovered peptide hormone that appears to be implicated in the mechanisms leading to preeclampsia, independently of angiogenic factors. The aim of the current study was to investigate whether women with early- or late-onset preeclampsia have altered ELABELA plasma concentrations compared to gestational-age-matched normal pregnant women.

Methods: This retrospective cross sectional study focused on the maternal plasma samples collected from 236 women with a singleton pregnancy who were allocated into the following groups: 1) early-onset preeclampsia (<34 weeks of gestation, N=56); 2) late-onset preeclampsia (≥ 34 weeks of gestation, N=57); and 3) gestational-age-matched controls with a normal pregnancy [(<34 weeks of gestation, N=59); (≥ 34 weeks of gestation, N=60)]. ELABELA plasma concentrations were determined using a validated enzyme immunoassay.

Results: 1) ELABELA plasma concentrations are higher in patients with late-onset preeclampsia compared to those from gestational-age-matched controls with a normal pregnancy [median: 7.99 ng/mL (IQR, 3–11.19 ng/mL) vs. median: 4.17 ng/mL (IQR, 5.3–13.95 ng/mL), $p=0.001$]; 2)

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DECLARATION OF INTEREST STATEMENT

The authors report no conflicts of interest.

ELABELA plasma concentrations in patients with early-onset preeclampsia do not differ from those of normal pregnant women [median: 6.09 ng/mL (IQR, 2.8–10.66 ng/mL) vs. median: 4.02 ng/mL (IQR, 3.26–7.49), $p=0.32$]; and 3) ELABELA plasma concentrations are higher in patients with late-onset preeclampsia compared to those with early-onset preeclampsia [median: 7.99 ng/mL (IQR, 3–11.19 ng/mL) vs. median: 6.09 ng/mL (IQR, 2.8–10.66 ng/mL), $p=0.01$].

Conclusion: ELABELA plasma concentrations are higher in patients with late-onset preeclampsia than in those with a normal pregnancy. However, women with early-onset preeclampsia have similar ELABELA plasma concentrations to those with a normal pregnancy. These findings provide insight into the ELABELA axis during the human syndrome of preeclampsia. In addition, these data support the concept that different pathophysiologic mechanisms are implicated in early- and late-onset preeclampsia.

Keywords

ELA; Toddler; Apela; Placenta; Maternal Vascular Malperfusion; Maternal Vascular Underperfusion; Vascular Disease; Pregnancy; Preterm Delivery; Placental Pathology; Early-onset Preeclampsia; Placental Villi

Introduction

Preeclampsia is a frequent complication of pregnancy and, therefore, the leading cause of maternal [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18] and perinatal [1, 2, 8, 12, 19, 20, 21, 22, 23, 24] morbidity and mortality. Growing evidence suggests that preeclampsia is not a single disorder but rather a multi-etiological syndrome [12, 25, 26, 27]. Etiologies for this syndrome include abnormal placentation [28, 29, 30, 31], utero-placental ischemia [12, 32, 33, 34, 35, 36], vascular disorders of the placenta [37, 38, 39, 40], insulin resistance [41, 42, 43, 44, 45], systemic maternal inflammation [46, 47, 48, 49, 50, 51], endothelial dysfunction [52, 53, 54, 55, 56, 57], and an imbalance of angiogenic and anti-angiogenic factors [33, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81].

Preeclampsia can be subdivided into early-onset (<34 weeks of gestation) and late-onset (>34 weeks of gestation) variants [82, 83, 84], of which the latter is the most frequent [85, 86, 87]. The pathophysiology observed between early- and late-onset preeclampsia is different: in late-onset preeclampsia, the angiogenic/anti-angiogenic imbalance is milder than in the early-onset variant [62, 65, 70, 88, 89, 90, 91, 92, 93, 94]. Indeed, such factors [placental growth factor (PlGF), soluble vascular endothelial growth factor receptor-1 (or soluble fms-like tyrosine kinase-1, sFLT1), soluble endoglin] and their ratios are considered biomarkers for the detection of early-onset preeclampsia [62, 63, 71, 86, 88, 89, 95, 96, 97, 98, 99]. In addition, models combining maternal blood pressure, uterine artery Doppler, PlGF, pregnancy-associated plasma protein A, and inhibin-A had been developed for the prediction of preeclampsia [85, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110].

Recently, it was reported that Elabela (also known as Apela/Toddler/ElA), a peptide hormone that is an endogenous ligand for the apelin receptor [111, 112], is implicated in the pathophysiology of preeclampsia [113]. Specifically, Ho et al. showed that *Elabela*-deficient

mice had thin placental labyrinths with poor vascularization and increased apoptosis as well as delayed syncytiotrophoblast differentiation, suggesting that this hormone is required in the development of the placenta and embryo [113]. *Elabela*-deficient mice also displayed preeclampsia-like features, e.g., hypertension, proteinuria, glomerular endotheliosis, and low birthweight, all of which were rescued by exogenous administration of this hormone [113]. These features were not associated with a decreased PlGF/sFlt1 plasma ratio, suggesting that Elabela acts independently of these angiogenic factors in the pathogenesis of preeclampsia [113]. Importantly, ELABELA was localized in the villous cytotrophoblast and syncytiotrophoblast of human placentas [113]. However, whether ELABELA concentration is decreased in the circulation of women with preeclampsia is unknown.

The aim of the current study was to investigate whether women with early- or late-onset preeclampsia have altered ELABELA plasma concentrations compared to gestational-age-matched normal pregnant women.

Materials and methods

Study design

This retrospective cross sectional study comprised 236 women with a singleton pregnancy who were allocated into two groups: 1) patients with a normal pregnancy (N=119: <34 weeks of gestation, N=59; 34 weeks of gestation, N=60) and 2) patients who developed preeclampsia (N=113). According to gestational age at diagnosis, patients with preeclampsia were divided into early- (<34 weeks of gestation, N=56) and late-onset preeclampsia (34 weeks of gestation, N=57). The clinical characteristics of the study population are shown in Table 1. The exclusion criteria were: 1) known major fetal or chromosomal anomalies and 2) multiple gestations. All patients were enrolled at the Detroit Medical Center, Wayne State University, Center for Advanced Obstetrical Care and Research of the Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS). Plasma samples were retrieved from the Bank of Biological Materials of the Perinatology Research Branch. All patients provided written informed consent. The use of biological specimens was approved by the Wayne State University Human Investigation Committee and the Institutional Review Board of NICHD.

Clinical definitions

Women with a normal pregnancy met the following criteria: (1) no medical, obstetrical, or surgical complications at the time of the study; (2) gestational age ranging from 20 to 41 weeks; and (3) delivery of a term infant, appropriate for gestational age, without complications. Preeclampsia was defined as new-onset hypertension that developed after 20 weeks of gestation (systolic or diastolic blood pressure 140 and/or 90 mm Hg, respectively, measured on at least two occasions, 4 hours to 1 week apart) and proteinuria (> 300 mg in a 24-hour urine collection, or two random urine specimens obtained 4 hours to 1 week apart containing 1+ by dipstick or one dipstick demonstrating 2+ protein) [114]. Early-onset preeclampsia was diagnosed <34 weeks of gestation [82], while late-onset preeclampsia was diagnosed 34 weeks of gestation [82, 92, 115].

Histopathologic examination of the placenta

Histopathologic assessment of placental lesions was performed by the examination of hematoxylin and eosin-stained sections of the chorioamniotic membranes, placental disc, and umbilical cord, according to protocols established by the Perinatology Research Branch. Acute histologic chorioamnionitis was diagnosed based on the presence of inflammatory cells in the chorionic plate and/or chorioamniotic membranes [116, 117, 118, 119]. Acute funisitis was diagnosed by the presence of neutrophils in the wall of the umbilical vessels and/or Wharton's jelly, using previously described criteria [116, 117, 120, 121, 122, 123]. Placental lesions consistent with maternal vascular lesions of malperfusion were diagnosed using criteria established by the Perinatal Section of the Society for Pediatric Pathology [124, 125].

ELABELA enzyme immunoassay

Maternal plasma concentrations of ELABELA were determined using a sensitive and specific enzyme immunoassay (Peninsula Laboratories, San Carlos, CA), according to the manufacturer's instructions. The inter- and intra-assay coefficients of variation were 13.86% and 9.51%, respectively. The sensitivity of the assays was 0.088 ng/mL.

Statistical analysis

Distribution of the data was assessed with the Kolmogorov-Smirnov test. Mann-Whitney U tests were used to compare continuous variables between groups. Fisher's exact test was used for comparisons of categorical variables between groups. Statistical analysis was performed using the SPSS software version 19 (IBM Corporation, Armonk, NY). A p-value <0.05 was considered statistically significant.

Results

Characteristics of the study population

A total of 236 women were included in this study. Table 1 summarizes the demographic and clinical characteristics of the study population. Both patient groups (early- and late-onset preeclampsia) had a higher rate of nulliparity ($p<0.001$), higher median systolic and diastolic blood pressures ($p<0.001$), lower median gestational age at delivery ($p<0.001$), and lower median birthweight ($p<0.001$) than their counterparts with a normal pregnancy.

Patients with early-onset preeclampsia had a lower median body mass index ($p=0.04$) than women with an uncomplicated pregnancy. Patients with late-onset preeclampsia had a lower median maternal age at delivery ($p=0.008$) and a higher percentage of male infants ($p=0.006$) compared to their counterparts with a normal pregnancy.

Patients with early-onset preeclampsia had a higher rate of maternal vascular lesions of malperfusion in the placenta than their counterparts with a normal pregnancy [82.1% (46/56) vs. 18.6% (11/59), $p<0.001$; Table 1]. No statistical differences in the rate of maternal vascular lesions of malperfusion were observed between patients with late-onset preeclampsia and their counterparts with a normal pregnancy [43.9% (25/57) vs. 30% (18/60), $p=0.13$; Table 1].

ELABELA plasma concentrations in patients with early-onset preeclampsia

Patients with early-onset preeclampsia had a slightly higher median concentration of ELABELA than their counterparts with a normal pregnancy [median: 6.09 ng/mL (IQR, 2.8–10.66 ng/mL) vs. median: 4.02 ng/mL (IQR, 3.26–7.49), $p=0.32$; Figure 1]; yet, such an increment did not reach statistical significance.

ELABELA plasma concentrations in patients with late-onset preeclampsia

Patients with late-onset preeclampsia had a higher median concentration of ELABELA than their counterparts with a normal pregnancy [median: 7.99 ng/mL (IQR, 3–11.19 ng/mL) vs. median: 4.17 ng/mL (IQR, 5.3–13.95 ng/mL), $p=0.001$; Figure 2]. Additionally, patients with late-onset preeclampsia had a higher median concentration of ELABELA than those with early-onset preeclampsia [median: 7.99 ng/mL (IQR, 3–11.19 ng/mL) vs. median: 6.09 ng/mL (IQR, 2.8–10.66 ng/mL), $p=0.01$; comparison not shown].

Discussion

Principal findings of the study

In the current study, we report that ELABELA plasma concentrations are higher in patients with late-onset preeclampsia compared to those from gestational-age-matched controls with a normal pregnancy or those with early-onset preeclampsia. However, ELABELA plasma concentrations in patients with early-onset preeclampsia did not differ from those of gestational-age-matched controls with a normal pregnancy.

Increased ELABELA plasma concentrations in women with late-onset preeclampsia

ELABELA is a conserved peptide present in all vertebrates and is encoded by the *ELABELA* gene, which was initially considered to be non-coding [111]. This hormone peptide competes with apelin for the apelin receptor [111, 112]. ELABELA is involved in embryogenesis by promoting mesoderm and angioblast migration during zebrafish development [112, 126]. The silencing of *ELABELA* causes zebrafish embryos to develop a rudimentary or no heart, a phenotype similar to that caused by the deletion of the apelin receptor, suggesting that the ELABELA/apelin receptor pathway is essential for the proper differentiation of the endoderm and heart morphogenesis in zebrafish [111, 127].

In adult mice, Elabela exerts its effects in the heart by increasing cardiac contractility, ejection fraction, and cardiac output and by inducing vasodilation via the apelin receptor [128, 129, 130]. In a mouse model of pressure overload-induced heart failure, infusion of Elabela improves cardiac dysfunction, hypertrophy, and fibrosis [131]. Such a protective effect is mediated via the suppression of the angiotensin-converting enzyme and pathogenic angiotensin II signaling [131]. Exogenous administration of Elabela has also been shown to exhibit cardiovascular protective effects in a monocrotaline rat model of pulmonary arterial hypertension [130] and an experimental rat model of septic shock [132]. Together, these reports provide solid evidence supporting a cardiovascular protective effect for ELABELA.

In the study herein, women with late-onset preeclampsia had higher ELABELA plasma concentrations than those presenting the early-onset variant. Women with late-onset

preeclampsia have a lower total vascular resistance and higher cardiac output than those with early-onset preeclampsia [101]. In addition, women with late-onset preeclampsia are at a lower risk for death by cardiovascular disease later in life than those with early-onset preeclampsia [133, 134]. Taken together, these data suggest that women with late-onset preeclampsia have high plasma ELABELA concentrations as a protective mechanism against the elevated cardiac output observed during this syndrome which, may translate into a lower risk for developing cardiovascular disease later in life. Additionally, these data support the concept that early- and late-onset preeclampsia differ in their respective pathophysiologies [70, 89, 101, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146].

In the current study, however, we did not find differences in the ELABELA plasma concentrations between women with early-onset preeclampsia and those with a normal pregnancy. This is contrasting with the fact that *Elabela*-deficient mice develop an early preeclampsia-like syndrome [113]. Further investigation is needed in order to determine whether women with early-onset preeclampsia have reduced expression of ELABELA at the placental level.

A previous report showed that ELABELA was mostly undetected in the plasma of women during the first trimester [147]. Yet, we were able to detect ELABELA in the plasma of women during the second and third trimester. These data are consistent with a prior study indicating that ELABELA is easily detectable in the plasma of healthy individuals (women and men) [130]. Indeed, ELABELA plasma concentrations in healthy humans [130] are lower than those reported herein (women with a normal pregnancy), which is consistent with the demonstration that pregnant dams have higher concentrations of this hormone peptide than non-pregnant mice [113].

Possible mechanisms of action of ELABELA during human pregnancy

In humans, ELABELA is expressed in embryonic [111, 148] and induced pluripotent stem cells [148] and cardiovascular tissues [130], as well as in the kidney [111, 148], prostate [111], lung [130], and placenta (villous cytotrophoblast and syncytiotrophoblast from the first trimester and term gestation) [113]. Additionally, the mature peptide of ELABELA is detected in human blood from healthy individuals [130] but does not co-localize with Weibel-Palade bodies [130], suggesting that such a peptide is constitutively present in circulation rather than a pregnancy-induced hormone.

Recently, it was proposed that ELABELA is produced by the trophoblast to prevent gestational hypertension as follows: 1) ELABELA, through its paracrine effects on fetal endothelial cells, enables angiogenesis and the formation of an adequate labyrinth network required for a proper perfusion; and 2) ELABELA reaches the maternal circulation in order to regulate cardio-renal function through its effects on the maternal endothelium [130] or by controlling fluid balance [149]. Additionally, it was speculated that ELABELA participates in placentation by regulating trophoblast migration and invasion [113]. Such protective effects of pregnancy were most likely mediated through the apelin receptor signaling [113].

Consistent with this hypothesis, in murine mid-pregnancy, *Elabela* serum concentrations are higher than in a non-pregnant state and its deficiency causes preeclampsia-like symptoms

[113]. Therefore, it will be expected that ELABELA concentrations will be lower in women with preeclampsia than in those with a normal pregnancy. The data presented herein show that ELABELA plasma concentrations in women with late-onset, but not early-onset, preeclampsia are increased compared to gestational-age-matched controls with normal pregnancies. In line with our findings, a previous report demonstrated that apelin and the apelin receptor are increased in the placenta (cytotrophoblast, syncytiotrophoblast, and stroma) from women with late-onset preeclampsia compared to those from women with a normal pregnancy [150]. Further research is required in order to investigate whether the entire ELABELA axis (ELABELA, apelin, and apelin receptor) is altered in the circulation and placentas of women with early- and late-onset preeclampsia.

In conclusion, the data presented herein show that ELABELA plasma concentrations are higher in patients with late-onset preeclampsia than in women with a normal pregnancy. However, women with early-onset preeclampsia have similar ELABELA plasma concentrations than those with a normal pregnancy. These findings provide evidence that the circulating levels of ELABELA in women with late-onset, but not early-onset, preeclampsia are dysregulated. In addition, these data support the concept that different pathophysiologic mechanisms are implicated in early- and late-onset preeclampsia.

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Figure 1. Plasma ELABELA concentration (ng/mL) in women with a normal pregnancy (n=59) and women with early-onset preeclampsia (n=56). Red line represents the median.

Figure 2. Plasma ELABELA concentration (ng/mL) in women with a normal pregnancy (n=60) and women with late-onset preeclampsia (n=57). Red line represents the median.

Table 1.

Demographic and clinical characteristics of the study population

	Normal pregnancy (<34 weeks)	Early-onset preeclampsia (<34 weeks)	P-value	Normal pregnancy (34 weeks)	Late-onset preeclampsia (34 weeks)	P-value
N	59	56		60	57	
Maternal age (years) ^a	24 (21–28)	24 (19.25–28.75)	0.459	25 (22–29)	22 (19.5–27)	0.008
Body mass index (kg/m ²) ^a	31.35 (25.17–35.25)	26.6 (23–32.3)	0.04	28.5 (23.7–34.17)	27.4 (23–33.95)	0.272
Race ^b			0.038			0.546
African-American	96.6% (57/59)	83.9% (47/56)		86.7% (52/60)	91.2% (52/57)	
Caucasian	0/59	8.9% (5/56)		8.3% (5/60)	3.5% (2/57)	
Other	3.4% (2/59)	7.1% (4/56)		5% (3/60)	5.4% (3/57)	
Nulliparous ^b	5.1% (3/59)	33.9% (19/56)	<0.001	10% (6/60)	43.9% (25/57)	<0.001
Smoking ^b	23.7% (14/59)	28.6% (16/56)	0.672	28.3% (17/60)	5.3% (3/57)	0.001
Systolic blood pressure (mmHg) ^a	127 (118–136)	177.5 (164.5–189.5)	<0.0001	128.5 (120–141.8)	158 (145–171)	<0.0001
Diastolic blood pressure(mmHg) ^a	73 (67–81)	109 (100.3–115)	<0.0001	74 (64–80)	101 (93.5–110.5)	<0.0001
Delivery ^b			<0.001			<0.001
Vaginal	(0/59)	30.4% (17/56)		0	77.2% (44/57)	
C/S	100% (59/59)	69.6% (39/56)		100% (60/60)	22.8% (13/57)	
Sex ^b			1			0.006
Female	40.7% (24/59)	39.3% (22/56)		63.3% (38/60)	36.8% (21/57)	
Male	59.3% (35/59)	60.7% (34/56)		36.7% (22/60)	63.2% (36/57)	
Birthweight (grams) ^a	3250 (3025–3459)	1327 (990–1760)	<0.001	3265 (3120–3573.75)	2955 (2712.5–3290)	<0.001
Percentile ^a	44.9 (28.7–59)	22.3 (15.2–31.22)	<0.001	40.3 (32.57–65.15)	36.7 (22.2–53.3)	0.033
APGAR 1 ^a	8 (8–9)	7 (5–8)	<0.001	9 (8–9)	8 (8–9)	0.28
APGAR 5 ^a	9 (9–9)	8 (8–9)	<0.001	9 (9–9)	9 (9–9)	0.864
Gestational age at sampling (weeks) ^a	30.3 (27.4–32)	30.45 (27.75–31.97)	0.867	37.35 (36.4–38.77)	37.3 (36.4–38.65)	0.983

	Normal pregnancy (<34 weeks)	Early-onset preeclampsia (<34 weeks)	P-value	Normal pregnancy (34 weeks)	Late-onset preeclampsia (34 weeks)	P-value
Gestational age at delivery (weeks) ^a	39 (38.6–39.4)	30.8 (28.45–32.32)	<0.001	39.1 (38.9–39.4)	37.6 (36.95–39)	<0.001
Histologic placental findings						
Acute histologic chorioamnionitis ^b	3.4% (2/59)	0	0.496	0	1.8% (1/57)	0.487
Acute funisitis ^b	5.1% (3/59)	0	0.244	3.3% (2/60)	10.5% (6/57)	0.156
Maternal vascular lesions of malperfusion ^b	18.6% (11/59)	82.1% (46/56)	<0.001	30% (18/60)	43.9% (25/57)	0.13

Data are given as median (interquartile range) and percentage (n/N)

^a Mann-Whitney U test.

^b Fisher's exact test.