ARAŞTIRMA / RESEARCH

Maternal plasma Elabela levels in intrauterine growth restriction

İntrauterin gelişme geriliğinde maternal serum Elabela düzeyleri

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Abstract

Purpose: The aim of this study was to investigate the role of Elabela in the etiopathogenesis of intrauterine growth retardation (IUGR).

Materials and Methods: The present prospective study included 43 healthy pregnant women and 46 pregnant women with IUGR. Maternal serum were collected from the control group and IUGR group at time of delivery. Elabela levels were analyzed in maternal serum through ELISA method.

Results: Lower Elabela levels (IUGR: 1.09 (0.61-15.61) ng/mL and Control: 1.44 (0.37-31.33) ng/mL ) were observed in IUGR patients compared to controls. Elabela levels were positively correlated with gestational week and neonatal weight. (r = 0.263, p = 0.013 and r = 0.259, respectively; p = 0.014).

Conclusion: The present study serves as the first study revealing the role of the decrease in Elabela in maternal serum on the etiopathogenesis of IUGR. This suggests that Elabela could be a potential drug target in cases with IUGR.

Keywords: Intrauterine growth restriction; Elabela; Pregnancy

INTRODUCTION

Intrauterine growth restriction (IUGR) is a fetal development disorder in which the fetus weight is <10th percentile expected for gestational age. The incidence of IUGR has been reported to be between 7-10% of all pregnancies¹. When there is IUGR, the fetus does not reach the defined growth potential. Of all the measurements used to calculate the estimated fetal weight (abdominal circumference, biparietal diameter, femur length), abdominal circumference is the most sensitive parameter for the estimation of IUGR. In addition, no other marker for IUGR applied during serial biometry monitoring once every 2-3 weeks, can show the estimated growth rate.

Other parameters used to support the diagnosis of IUGR, and to monitor the health of the fetus and the timing of delivery include a reduction in amniotic fluid determined sonographically and/or deterioration in Doppler parameters (loss of end-diastolic flow in umbilical artery/reverse flow, reverse wave in ductus venosus, cerebroplacental...
index of <1.08.3,5 The reasons for IUGR may be maternal (smoking, autoimmune diseases, pre-gestational DM, insufficient nutrition), fetal (aneuploidy, intrauterine infections, structural abnormalities, genetic syndromes) and placental (ablatio placenta, environmental placenta, velamentous cord placement).6 However, these factors are only seen in 30% of all IUGR cases. The remaining 70% of cases are defined as idiopathic and are based on a common pathology on the basis of weak uteroplacental perfusion leading to insufficient fetal nutrition and growth restriction7. Normal fetal development is based on the process known as re-modelling, primarily the invasion of uterus spiral arterioles by trophoblasts and the transformation to low-resistant placental vessels. It has been suggested that maternal vascular endothelial dysfunction and insufficient remodelling in the spiral artery during placentation play a role in the etiopathogenesis of IUGR.6

Elabela (also known as Toddler or Apela) is a peptide hormone which binds to the apelin receptor (APLNR) bound to G-protein7. Elabela has been defined as localised in the endothelium of the adult heart and blood vessels and affinity to APJ receptor has been reported. Elabela has also been determined in human stem cells, and in the prostate and kidneys7,8. Previous studies have shown that APJ receptor is expressed at high rates in both the endothelial cells and angioblasts of vessels developing in the embryos of mice, frogs, and zebra fish9-11. The most recent studies have shown that the Elabela-APJ axis plays a role in vasculogenesis, and the apelin-APJ axis plays a role in embryonic angiogenesis10-12.

It has been demonstrated that Elabela in human placenta is expressed in cytotrophoblasts and syncytiotrophoblasts throughout pregnancy13. The addition of exogenous Elabela has been reported to increase trophoblast invasiveness using choriocarcinoma cells which are similar to human trophoblasts, and by Elabela supporting the invasion of trophoblasts to the maternal uterus wall, the formation of early placenta is supported. Accordingly, it has been emphasised that mice deficient in Elabela show thinner placental labyrinths (feto-maternal exchange area) and smaller placenta than control mice. Weak vascularisation characterised by less angiogenic germination has been observed in the placenta of mice defective in Elabela13 and this suggests that placenta angiogenesis is supported by Elabela. To the best of our knowledge, there is no study in literature that has evaluated maternal serum Elabela levels in IUGR cases. Therefore, the aim of this study was to evaluate the role of Elabela in idiopathic IUGR cases.

MATERIALS AND METHODS

Study population

This prospective study, conducted between May 2019 and May 2020, included 46 patients with unexplained IUGR and 43 healthy pregnant patients. Gestational age was confirmed on the ultrasound taken in the first trimester. IUGR was defined as infant birthweight <10th percentile for index gestational age. All study group subjects had a pulsatility index of 95% or higher for fetal umbilical artery Doppler measurements, showing impaired placental blood flow. Patients were excluded from the study if they had any chronic disease including kidney or liver dysfunction, if they smoked, had a multiple pregnancy, pre-eclampsia, acute or chronic inflammatory disease, congenital fetal infection, or any fetal structural or chromosomal abnormalities. The volunteers in the control group had no history of disease before or during the pregnancy.

This cross-sectional study was conducted in the Obstetrics and Gynaecology Clinic and the Medical Biochemistry Laboratory of Kahramanmaraş Sütçü Imam University Medical Faculty. Approval for the study was granted by the Clinical Research Ethics Committee of Kahramanmaraş Sütçü Imam University (03.04.2019/18). Informed consent was provided by all the study participants.

In a study of patients with pre-eclampsia, Deniz et al.14 detected a 95.1% difference in Elabela level between pre-eclamptic patients and healthy individuals. According to the results of this study, the power analysis with a test power to be 0.95 by accepting the error as 0.05 required the inclusion of at least 8 patients in consideration of the rate of 95.1% in terms of Elabela levels.

Following physical and ultrasound examination, a peripheral blood sample of 1 tube was taken from all the patients for analysis of Elabela levels. The samples were centrifuged at 4000 rpm for 10 min, then after separation of the serum, the samples were stored at -80°C until assay. Body mass index (BMI) was calculated as bodyweight (kg)/height squared (m²).
**Serum MOTS-C Measurements**

Human serum Elabela concentration was analysed using the enzyme-linked immunosorbent assay (ELISA) method with commercial kits (Shanghai Sunred Biological Technology Co., Ltd, China, Cat. No: 201-12-8569) as per the manufacturer’s instructions. In the Elabela analysis, the intra-assay variation coefficient was <10%, and the inter-assay, <12%.

The measurements were taken automatically on the ELISA reader (ThermoScientific, Finland) and using a computer program (Scanlt for Multiscan FC 2.5.1). The absorbancy of each well was determined as 450nm. The standard curve was drawn as mean absorbency of standards (Y) and known concentration of standards (X). The results were reported as the Elabela concentration (ng/mL) in the samples.

**Statistical analysis**

Data obtained in the study were analysed statistically using SPSS vn. 20.0 for Windows software (SPSS, Inc., Chicago, IL, USA). Of the demographic data, categorical values were stated as number (n) and percentage (%), and numerical variables as median (minimum-maximum) values. Conformity of the data to normal distribution was assessed with the Kolmogorov-Smirnov test and the Shapiro-Wilk test. In the comparison of categorical data with numerical data, the Mann Whitney U-test was applied. Spearman correlation analysis was used in the evaluation of relationships between the data. The level of correlation was interpreted by examining the correlation coefficient (r value). A ROC curve was applied to determine the diagnostic value of Elabela. A value of p<0.05 was accepted as statistically significant.

**RESULTS**

The demographic data are shown in Table 1. The serum levels of Elabela were detected to be significantly lower in the study group compared with the control group (IUGR: 1.09 (0.61-15.61) ng / mL and Control:1.44 (0.37-31.33) ng / mL p=0.031). (Figure 1). No significant difference was determined between the groups in respect of gravidity, parity, number of living children and gender (p>0.05). Correlations between maternal data and neonatal outcomes were examined. A positive correlation was determined between serum Elabela level and gestational week and neonatal weight (r=0.263; p=0.013 and r=0.259; p=0.014) (Figure2).

**Table 1. Demographic characteristics of pregnant women and newborns participating in the study**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study Group (n=46)</th>
<th>Control Group (n=43)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>27.5 (18-43)</td>
<td>30 (22-46)</td>
<td>0.171</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>160 (140-170)</td>
<td>162 (149-174)</td>
<td>0.042</td>
</tr>
<tr>
<td>Maternal weight (kilogram)</td>
<td>73.5 (49-93)</td>
<td>73 (56-95)</td>
<td>0.090</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>28.1 (19.4-38.7)</td>
<td>27.7 (20.8 -34.7)</td>
<td>0.090</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)*</td>
<td>35.6 (24.5-41.1)</td>
<td>38.5 (36.0-41.3)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Neatnatal birthweight (grams)*</td>
<td>1990 (550-3100)</td>
<td>3240 (2620-4000)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Apgar 1*</td>
<td>7 (5-9)</td>
<td>8 (7-9)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Apgar 5*</td>
<td>8 (6-10)</td>
<td>9 (8-10)</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Values were expressed as median (minimum-maximum) values; *p <0.05 was statistically significant.; BMI; Body Mass Index
Apgar 1; First minute Apgar Score; Apgar 5; Fifth minute Apgar Score

**Table 2. Receiver operating characteristics (ROC) curve analysis of the study parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elabela</td>
<td>0.633</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.584</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>0.473</td>
</tr>
<tr>
<td>Gravida</td>
<td>0.485</td>
</tr>
<tr>
<td>Parity</td>
<td>0.479</td>
</tr>
</tbody>
</table>

BMI; Body Mass Index
The ROC curve was drawn for serum Elabela. Low values indicated the presence of IUGR, with a cut-off point of 1.39, sensitivity of 0.696, and specificity of 0.535 for Elabela. The area under the curve (AUC) was found to be 0.633 (confidence interval, (CI) 95%: 0.517–0.749, p<0.05) for Elabela. (Figure 3). (Table 2)

**DISCUSSION**

IUGR is one of the most important causes of fetal and neonatal morbidity and mortality. Although there are many maternal, fetal and placental factors underlying the development mechanism of IUGR, the main pathogenesis is poor uteroplacental perfusion. Using mice models, Ho et al showed that Elabela deficiency resulted in placental dysfunction characterised by thin labyrinths and delayed syncytiotrophoblast differentiation in addition to poor vascularisation, increased apoptosis, and reduced proliferation. In the current study, maternal serum Elabela levels were compared in IUGR and healthy pregnancies, and the serum Elabela concentration was determined to be statistically significantly reduced in the IUGR patients. Elabela is of critical importance in early placental development. In early pregnancy, Elabela is produced
by villous cytotrophoblasts and syncytiotrophoblasts. It has been shown that Elabela supports cytotrophoblast proliferation, trophoblastic invasion to the maternal decidua and fetal vascular angiogenesis \textsuperscript{16}. By activating APJ receptor, Elabela has been shown to dilate the aorta in mice in an endothelium-dependent manner. In addition to findings such as poor placental vascularisation, low birthweight, hypertension and proteinuria seen in the Elabela-deficient mice, it was emphasised that no improvement was observed following administration of Elabela \textsuperscript{13}. This study also suggests that low Elabela levels cause IUGR development through insufficient vasodilatation and insufficient trophoblast invasion in the placental vessels.

In a previous study it was shown that exogenous administration of Elabela peptide could increase the invasiveness of extravillous trophoblasts in vitro, change the growth morphology, and reduce ex vivo trophoblast proliferation. However, it was emphasised that Elabela levels in circulation in women are extremely variable, they are correlated with BMI, and are significantly reduced in the plasma in the first trimester of women with a healthy BMI who develop pre-eclampsia \textsuperscript{17}. With the consideration that BMI affects the maternal serum Elabela level, the IUGR and control groups of the current study were formed of subjects with similar BMI values to eliminate this effect. There was no difference between the groups in respect of BMI and there was no correlation between Elabela levels and BMI.

In a study of late onset pre-eclampsia, a decrease was observed in mRNA and protein expression of Elabela and apelin receptor (APLNR or APJ) in placental tissues. It has been shown that the changed level of Elabela expression in the circulation could be a reflection of the changes in placental Elabela expression and are correlated with late onset pre-eclampsia, which can be used as a reference for diagnosis \textsuperscript{18}. In contrast, Elabela plasma concentrations of women with late onset pre-eclampsia have also been found to be higher than those of women with normal healthy pregnancies \textsuperscript{19}. Another study stated that protein transcription could be reduced in women expressing rare variants of the Elabela gene, and it was suggested that this could cause an increased risk of pre-eclampsia \textsuperscript{20}.

The results of the current study showed a moderate level correlation between Elabela values and the birthweight of infants born with IUGR. In a study of pre-eclampsia patients, Deniz et al determined lower maternal Elabela levels in those with pre-eclampsia compared to the women with healthy pregnancies, and a moderate level correlation was found between the Elabela levels and the birthweights of infants born to pre-eclamptic and severely pre-eclamptic mothers \textsuperscript{14}.

In the development of normal placenta, extravillous trophoblasts invade the muscle layer where the spiral arteries are found. This remodelling of the spiral arteries transforms them to high-capacity utero-placental vessels. Thus the spiral arteries provide sufficient blood flow for the developing fetus and the placenta \textsuperscript{21}.

In IUGR cases, there is known to be insufficient placentation, in other words, in trophoblast migration there is no invasion of the muscle layer where the spiral arteries are, and this creates an area with high resistance to blood flow, and as a result, reduced feeding of the intervillous area and reduced vasoconstrictor agent activity, which has potentially increased. In abnormal placentation, trophoblast migration of the myometrial section of the spiral arteries does not occur \textsuperscript{22}. As the spiral arteries have not developed in IUGR cases and because of insufficient blood flow to the fetus and the placenta, infants are born with a low birthweight because of insufficient nutrition of the fetus. Another reason for neonatal birthweight in cases with IUGR may be related to an insufficient amount of Elabela.

Chang et al and Wang et al associated low Elabela with the low birthweight of infants born to mothers with pre-eclampsia \textsuperscript{7,9}. Elabela is synthesised by the placenta \textsuperscript{13} and induces angiogenesis in the human cord vascular endothelial cells \textsuperscript{16}. Therefore, low birthweight in IUGR cases may be associated with insufficient angiogenesis in the placenta as a result of insufficient Elabela levels. If the Elabela levels are sufficient in the maternal circulation and placenta, this allows sufficient angiogenesis and thus by taking oxygen the fetus is fed with more blood (with more nutrients) and cannot be born at a low birthweight. In conclusion, this is the first study to have shown that the Elabela amount is reduced in the maternal blood of IUGR cases.

The measured serum Elabela level has been found to be significant with ROC analysis in respect of diagnostic value. In the ROC analysis of the current study, the cutoff value for Elabela was found to be 1.39 for the differentiation between the IUGR and control groups. Based on the findings of the current
study, Elabela modulation can be considered as a potential therapeutic strategy for IUGR treatment. One of the limitations of our study is that it was a single-center study with a small sample size. Interpretation and generalization of results should be done with caution. Furthermore, our data cannot confirm a definitive causal relationship between Elabela and angiogenesis. More animal studies are needed to evaluate the effect of Elabela on angiogenesis in vivo. Another limitation is that the measurements are made once, at the time of birth. The study can be strengthened by measurements to be made in the blood taken during the first diagnosis of pregnancy and cord blood.

The role that Elabela plays in pregnancy and in the mechanism of IUGR development remains unclear. This is the first study to have shown that reduced Elabela in the maternal serum has a role in the mechanism of IUGR development. Nevertheless, the results of this study suggest that Elabela could be a potential drug target in IUGR.

Yazar Katkıları: Çalışma konsenti/ Tanınım: FAB; Veri toplama: FAB, SK; Veri analizi ve yorumlama: FAB, SK; Yazi taslîği: FAB, SK; İşçilere ekstremite incelemleri: FAB, SK; Son onay ve sonuçları: FAB, SK; Teknik ve malzeme desteği: FAB, SK; Süpervizyon: FAB, SK; Fon sağlama (mevcut ise): yok.

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REFERENCES


