Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2021;46(4):1344-1350
DOI: 10.17826/cumj.976828

ARAŞTIRMA / RESEARCH

Maternal plasma Elabela levels in intrauterine growth restriction

İntrauterin gelişme geriliğinde maternal serum Elabela düzeyleri

Filiz Alkan Baylan[1]; Selim Karaküçük[2]

[1]Kahramanmaraş Sütcü İmam University Hospital, Department of Biochemistry, [2]Department of Obstetrics and Gynecology, Kahramanmaras, Turkey

Cukurova Medical Journal 2021;46(4):1344-1350

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

Öz

Amaç: Bu çalışmanın amacı, Elabela'nın intrauterin büyüme geriliğinin (IUGR) etyopatogenezindeki rolünü araştırmaktı.

Gereç ve Yöntem: Bu prospektif çalışmaya 43 sağlıklı gebe ve 46 IUGR'li gebe dahil edildi. Kontrol grubundan ve IUGR grubundan doğum sırasında anneden kan alındı. Elabela seviyeleri anne serumunda ELISA yöntemi ile analiz edildi.

Bulgular: Kontrollere kıyasla IUGR hastalarında daha düşük Elabela seviyeleri (IUGR: 1.09 (0.61-15.61) ng/mL ve Kontrol: 1.44 (0.37-31.33) ng/mL) gözlendi. Elabela seviyeleri, gebelik haftası ve yenidoğan ağırlığı ile pozitif korelasyon gösterdi. (sırasıyla r = 0.263, p = 0.013 ve r = 0.259; p = 0.014).

Sonuç: Bu çalışma, anne serumunda Elabela'daki azalmanın IUGR etyopatogenezindeki rolünü ortaya koyan ilk çalışma olma özelliğini taşımaktadır. Bu, Elabela'nın IUGR'li vakalarda potansiyel bir ilaç hedefi olabileceğini düşündürmektedir.

Anahtar kelimeler: İntrauterin büyüme geriliği; Elabela; Gebelik

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 pregnancies[1]. 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

Yazışma Adresi/Address for Correspondence: Dr. Filiz Alkan Baylan, Kahramanmaras Sütcü İmam University Hospital, Department of Biochemistry, Kahramanmaras, Turkey E-Mail: drfilizalkan@gmail.com
Geliş tarihi/Received: 31.07.2021 Kabul tarihi/Accepted: 10.09.2021 Çevrimiçi yayın/Published online: 16.09.2021

index of <1.08)[2,3]. 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)[4].

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 restriction[5]. 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-protein[7]. 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 kidneys[7,8]. 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 fish[9-11]. 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 angiogenesis[10-12].

It has been demonstrated that Elabela in human placenta is expressed in cytotrophoblasts and syncytiotrophoblasts throughout pregnancy[13]. 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 Elabela [13] 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 structual 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 (m^2).

Serum MOTS-C Measurements

Human serum Elabela concentration was analysed using the ennzyme-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 coeffficient (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 gravida, 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

	Study Group (n=46)	Control Group (n=43)	p Value
Maternal age (years)	27.5 (18-43)	30 (22-46)	0.171
Maternal height (cm)	160 (140-170)	162 (149-174)	0,042
Maternal weight (kilogram)	73,5 (49-93)	73 (56-95)	0.690
Maternal BMI	28.1 (19.4-38.7)	27.7 (20.8 -34.7)	0,690
Gestational age at birth (weeks)*	35.6 (24.5-41.1)	38.5 (36.0-41.3)	0.000*
Neonatal birthweight (grams)*	1990 (550-3100)	3240 (2620-4000)	0.000*
Apgar 1*	7 (5-9)	8 (7-9)	0.000*
Apgar 5*	8 (6-10)	9 (8-10)	0.000*

Values were expressed as median (minumum-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

Parameters	AUC
Elabela	0.633
Maternal age	0.584
Maternal BMI	0.473
Gravida	0.485
Parity	0.479

BMI; Body Mass Index

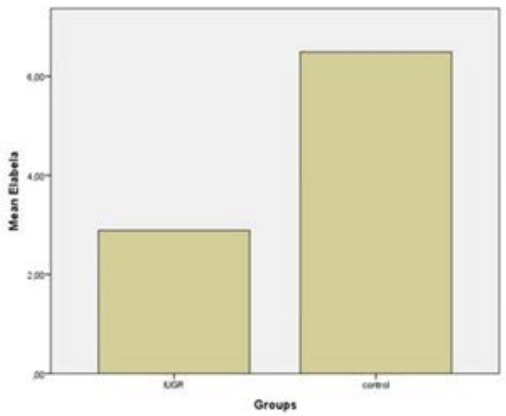

Figure 1. Serum Elabela levels (ng/Ml) of study groups.

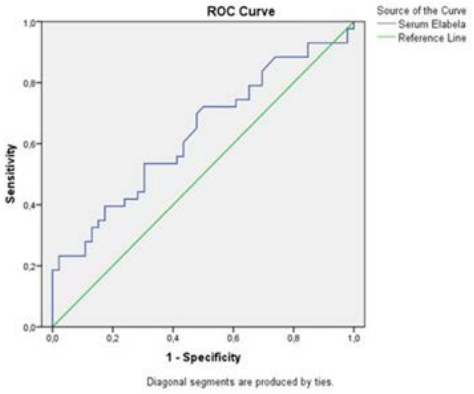

Figure 3. ROC curve of Elabela.

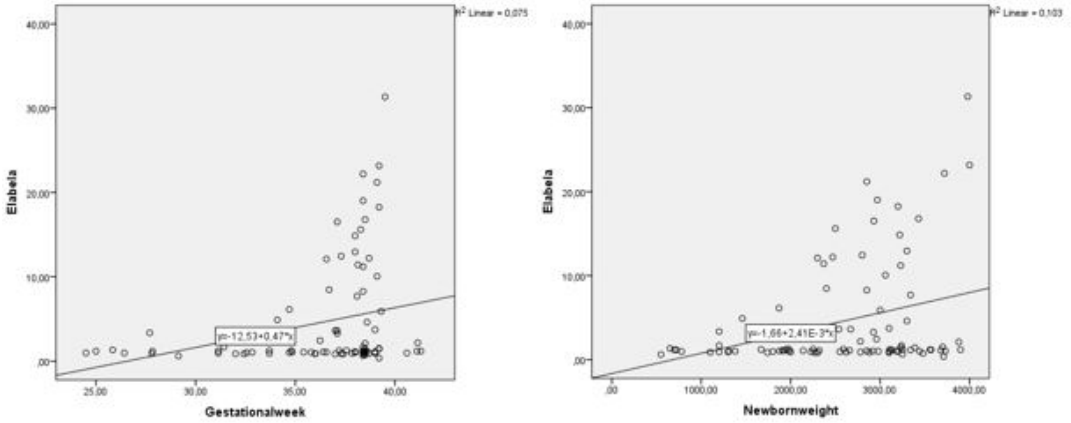

Figure 2. Correlation between Elabela levels and gestational week and neonatal weight.

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 [15]. 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 [13]. 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 [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 [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 extravıllous 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 [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 [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 [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 [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 [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 [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 ocur [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 asociated low Elabela with the low birthweight of infants born to mothers with pre-eclampsia [7,8]. Elabela is synthesised by the placenta [13] and induces angiogenesis in the human cord vascular endothelial cells [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 etiopathogenesis of IUGR, but there is a need for further experimental research and clinical evidence to clarify these mechanisms. Nevertheless, the results of this study suggest that Elabela could be a potential drug target in IUGR.

Yazar Katkıları: Çalışma konsepti/Tasarımı: FAB; Veri toplama: FAB, SK; Veri analizi ve yorumlama: FAB, SK; Yazı taslağı: FAB, SK; İçeriğin eleştirel incelenmesi: FAB, SK; Son onay ve sorumluluk: FAB, SK; Teknik ve malzeme desteği: FAB, SK; Süpervizyon: FAB, SK; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Kahramanmaraş Sütçü İmam Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulundan 03.04.2019 tarih ve 2019/06-18 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

Author Contributions: Concept/Design : FAB; Data acquisition: FAB, SK; Data analysis and interpretation: FAB, SK; Drafting manuscript: FAB, SK; Critical revision of manuscript: FAB, SK; Final approval and accountability: FAB, SK; Technical or material support: FAB, SK; Supervision: FAB, SK; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained for this study from Kahramanmaraş Sütçü İmam University Faculty of Medicine Clinical Research Ethics Committee with the decision dated 03.04.2019 and numbered 2019/06-18.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

REFERENCES

1. Figueras F, Gratacos E. Update on the diagnosis and classification of fetal growth restriction and proposal of a stage-based management protocol. Fetal Diagn Ther. 2014;36:86–98.
2. Hui L, Challis D. Diagnosis and management of fetal growth restriction: the role of fetal therapy. Best Pract Res Clin Obstet Gynaecol. 2008;22:139–58.
3. Sharma D, Shastri S, Farahbakhsh N, Sharma P. Intrauterine growth restriction–part 1. J Maternfetal Neonat Med. 2016;29:3977–87.
4. Kesavan K, Devaskar SU. Intrauterine growth restriction: postnatal monitoring and outcomes. Pediatr Clin North Am. 2019;66:403-23.
5. Suhag A, Berghella V. Intrauterine growth restriction (IUGR): etiology and diagnosis. Curr Obstet Gynecol Rep. 2013;2:102–11.
6. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. Am J Obstet Gynecol. 2006;195:40–9.
7. Wang Z, Yu D, Wang M, Wang Q, Kouznetsova J, Yang R et al. Elabela- apelin receptor signaling pathway is functional in mammalian systems. Sci Rep. 2015;5:8170.
8. Chng SC, Ho L, Tian J, Reversade B. ELABELA: a hormone essential for heart development signals via the apelin receptor. Dev Cell. 2013;27:672-80.
9. Devic E, Rizzoti K, Bodin S, Knibiehlern B, Audigier Y. Amino acid sequence and embryonic expression of msr/apj, the mouse homolog of Xenopus X-msr and human APJ. Mech Dev. 1999;84:199–203.
10. Helker C.S, Schuermann A, Pollmann C, Chng SC, Kiefer F, Reversadeet B et al. The hormonal peptide Elabela guides angioblasts to the midline during vasculogenesis. eLife 2015;4:e06726.
11. Kidoya H, Ueno M, Yamada Y, Mochizuki N, Nakata M, Yano T et al. Spatial and temporal role of the apelin/APJ system in the caliber size regulation of blood vessels during angiogenesis. EMBO J.. 2008;27:522–34.
12. Cox CM, Agostino SLD, Miller MK, Heimark RL, Krieget PA. Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. Dev Biol (Basel). 2006;296:177–89.
13. Ho L, van Dijk M, Chye STJ, Messerschmidt DM, Chng SC, Ong S et al. ELABELA deficiency promotes preeclampsia and cardiovascular malformations in mice. Science. 2017;357:707–13.
14. Deniz R, Baykus Y, Ustebay S, Ugur K, Yavuzkir Ş, Aydin S et al. Evaluation of Elabela, apelin and nitric oxide findings in maternal blood of normal pregnant women, pregnant women with pre-eclampsia, severe pre-eclampsia and umbilical arteries and venules of newborns. J Obstet Gynaecol. 2019;39:907-12.
15. American College of Obstetricians and Gynecologists. Fetal growth restriction. Obstetrics gynecol practice bulletin No. 134. Obstet Gynecol. 2013;121:1122–33.
16. Eberlé D , Marousez L, Hanssens S, Knauf C, Breton C, Deruelle P et al. Elabela and apelin actions in healthy and pathological pregnancies. Cytokine Growth Factor Rev. 2019;46:45-53.
17. Georgiadou D, Boussata S, Ranzijn WHM, Root LEA, Hillenius S, Bij de Weg JM et al. Peptide Hormone ELABELA enhances extravillous trophoblast differentiation, but placenta is not the major source of circulating ELABELA in pregnancy. Sci Rep. 2019;9:19077.

18. Zhou L, Sun H, Cheng R, Fan X, Lai S, Deng C. ELABELA, as a Potential diagnostic biomarker of preeclampsia, regulates abnormally shallow placentation via APJ. Am J Physiol Endocrinol Metab. 2019;316:773-81.
19. Panaitescu B, Romero R, Gomez-Lopez N, Pacora P, Erez O, Vadillo-Ortega F et al. ELABELA plasma concentrations are increased in women with late-onset preeclampsia. J Matern Fetal Neonatal Med. 2020;33:5-15.
20. Yang X, Bian Y, Wan J, Li L, Yang P, Zhao S et al. Variants in the 5-UTR of APELA gene in women with preeclampsia. Prenat Diagn. 2019;39:308–13.
21. Knöfler M, Haider S, Saleh L, Pollheimer J, Gamage TKJB, James J. Human placenta and trophoblast development: key molecular mechanisms and model systems. Cell Mol Life Sci. 2019;76:3479-96.
22. Nardozza LMM, Caetano ACR, Zamarian ACP, Mazzola JB, Silva CP, Marçal VM,, et al. Fetal growth restriction: current knowledge. Arch Gynecol Obstet. 2017;295:1061–77.