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Serum elabela levels in women with tubal ectopic pregnancy: A case-control study

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Abstract

Elabela, a circulating peptide hormone derived from the placenta, plays a crucial role in embryonic development and function of the human placenta during pregnancy. We aimed to investigate serum elabela levels in ectopic pregnancy. Sixty cases admitted to Emergency Department and Obstetrics and Gynecology Clinic were included in this case-control study. Thirty women with tubal ectopic pregnancy constituted Group 1 and 30 women with healthy pregnancy served as controls (Group 2). Blood samples were collected from all participants. Demographic and obstetric characteristics of the whole study cohort were also recorded. Levels of serum elabela were measured in each group by enzyme-linked immunosorbent assay . Age, gender, gestational age, obstetrics history, and body mass index of the groups were similar. Mean serum elabela levels were 7.52 ± 1.1 ng/mL and 7.05 ± 1.2 ng/mL in group 1 and group 2, respectively. There was no statistically significant difference between the two groups with respect to serum elabela levels (t=1.581, p=0.119). In the present study, serum elabela levels were not significantly different in women with ectopic pregnancies than healthy pregnancies. Since placenta is the primary source of elabela, this lack of difference might be due to the insufficient placental as well as fetal development in fallopian tubes.

Keywords: Ectopic pregnancy, emergency, serum Elabela

Introduction

Pregnancy taking place outside the uterine cavity is described as ectopic pregnancy. Ectopic pregnancy complicates up to 2% of all pregnancies in the United States, and is the primary cause of maternal death in the first trimester [1,2]. Pregnancy of unknown location is used to define any pregnancy during the first five weeks since the last menstrual period of a woman. During this phase of pregnancy, imaging studies are not of sufficient sensitivity to reveal where the embryo is implanted [3]. Embryo implantations outside of the uterine cavity pose a threat to the life of the pregnant woman, so it is an evident clinical need to find a method of early detection to prevent this untoward consequence. Several novel biochemical markers and imaging modalities have been proposed to detect ectopic pregnancy before life-threatening complications occur [4-6]. Thus, a method enabling early recognition of ectopic pregnancy is of crucial importance to prevent untoward outcomes.

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Elabela is a recently discovered placental peptide hormone that behaves as a ligand for APJ, a G-protein coupled receptor, also known as angiotensin receptor-like 1. APJ receptor is widely expressed in several tissues throughout the human body [7]. Apelin is another endogenous cognate ligand of APJ receptor, which was discovered earlier than elabela. Both apelin and elabela have a number of similar functions. Elabela-APJ axis constitutes a crucial pathway for cardiovascular development. In addition, this system also has significant biological actions, including embryonic development, skeletal development, angiogenesis, vascular morphogenesis [8], fluid homeostasis, among others [9-11].

Ho and colleagues suggested that the APJ-elabela pathway may be implicated in the pathophysiology of pre-eclampsia [12]. The authors demonstrated that elabela-deficient animals developed pre-eclamptic symptoms while the infusion of lacking hormone restored blood pressure to normal and alleviated other findings. Since this first report, several studies have mentioned higher than normal levels of elabola in women with late-onset preeclampsia [13,14]. However, not all studies reported an increased serum elabela levels in women with preeclampsia compared with healthy pregnancies [15]. In contrast to elabela levels, older ligand of APJ,

apelin, shows significant increases in early-onset preeclampsia [16].

To the best of our knowledge, there is no data in the literature regarding levels of elabela in women with ectopic pregnancy. Since ectopic pregnancy inevitably has abnormal placentation, and abnormal placentation is associated with lower than normal serum elabela levels, we hypothesized that women with ectopic pregnancies might have declined serum elabela levels. Possible changes in Elabela levels may facilitate the diagnosis of ectopic pregnancy. Thus, we aimed to conduct a case-control study in which serum elabela levels of women with radiologically proven ectopic pregnancy were compared with those of gestational age-matched healthy pregnancies.

Materials and Methods

This study was carried out at Department of Emergency and Department of Obstetrics and Gynecology of Fırat University between June 2018 and June 2019 after approval of the study protocol by the local ethics committee (IRB number: 2019/11-20). All participants of the study completed written informed consent forms during enrollment.

During the recruitment period, a total of 35 consecutive patients were hospitalized due to suspected ruptured ectopic pregnancy. The study group consisted of patients who were diagnosed as ectopic pregnancy as a result of Beta HCG, complete blood count (CBC) and ultrasonographic findings of adnexal mass and intraabdominal free fluid. Two women, whose diagnosis of ectopic pregnancy was not ascertained, and one who had pelvic free fluid but had pregnancy of unknown location were excluded from the study. Of the remaining patients, 30 accepted to join the study (Group 1). Thirty age and gestational-week-matched women with no discernible health problems, who applied during the study dates served as the control group (Group 2). 'The study groups were divided into groups as case-control studies. The study flowdiagram is depicted in Figure 1.

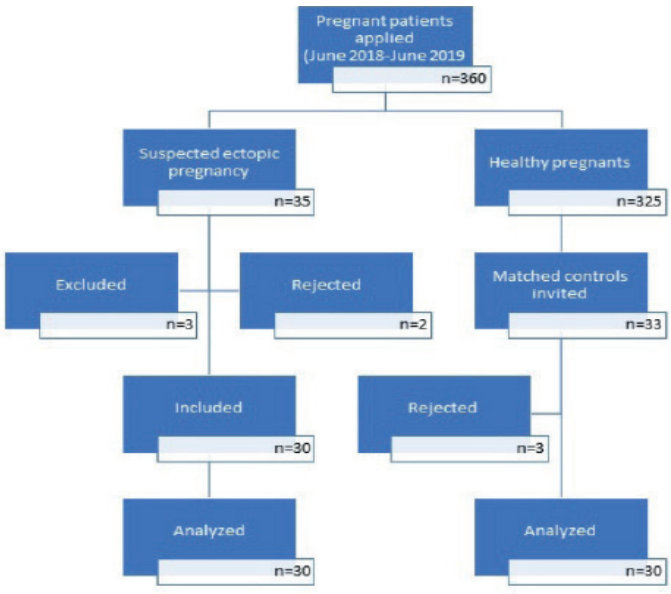

Figure 1. Study flow diagram

Variables

Demographic features and obstetric characteristics such as age, body mass index, pregnancy week, number of previous pregnancies, parity, and number of abortions were recorded for all study participants. In the study group, following clinical examination, two potential diagnoses were entertained: ectopic pregnancy or intrauterine abortion. To confirm the clinical suspicion of ectopic pregnancy, a transvaginal ultrasound was performed.

Five milliliters of blood was collected from the patients who gave consent for the study. Separated serums were stored at -70 C until analysis. The serum elabela level was measured via human Elabela enzyme-linked immunosorbent assay (ELISA) assay (Shanghai Sunred Biological Technology Co., Ltd, catalog no: 201-12-8569, China). Absorbances were measured spectrophotometrically at 450 nm in an ELISA micropellet reader (Thermo Scientific, Multiskan FC, USA). Results were reported as ng/mL. The assay sensitivity was 0.118 ng/ml, and the measurement interval was 0.15-40 ng/ml.

Study size

The sample size calculation was based on the main outcome variable serum elabela levels. Twenty-nine cases in each group (total n=58) are required to compare two means with μ1=7.0 ng/ml, μ2=7.75 ng/ml, and equal standard deviations of 1 (effect size=0.75, large) using the independent samples t-test taking, which will give a power of 80% at a two-tailed hypothesis [17].

Statistical methods

The normality check of continuous variables was performed with the Kolmogorov-Smirnov test. Independent-samples t-test was used to compare the numerical variables. SPSS 21.0 software package (IBM, Armonk, NY, USA) was used to analyze data derived from the study. A p-value lower than 0.05 was accepted as statistically significant.

Results

Demographic features, laboratory parameters and obstetric characteristics such as age, body mass index, pregnancy week, number of previous pregnancies, parity, and number of abortions were similar between the groups (Table 1).

Table 1. Demographic and obstetric characteristics of the study and control groups.

Table 1. Demographic and obstetric characteristics of the study and control groups

Variable	Group 1 (n=30)	Group 2 (n=30)	Significance
Age (years)	29.1±5.7	28.3±5.5	NS
Gestational Age (weeks)	5.5±0.6	5.6±0.7	NS
Number of Pregnancies (n)	2.5±1.3	2.2±1.0	NS
Parity (n)	1.2±0.9	1.0±0.9	NS
Number of Abortions (n)	0.3±0.5	0.1±0.3	NS
Body Mass Index	24.4±3.9	23.9±3.8	NS
Hemoglobin (g/L)	11.6±1.4	11.2±1.3	NS
Hematocrit (%)	34.4±3.6	33.7±3.5	NS
Beta-hCG (mIU / ml)	1859±213	1964±312	NS

NS: not statistically significant. Values are reported as mean ± Standard deviation. (p>0.05. Independent samples t-test).

Serum Elabela hormone levels were slightly higher in Group 1 compared to Group 2. However, this difference was not statistically different (t=1.581, p=0.119). The mean serum elabela levels were 7.52±1.1 ng/mL and 7.05±1.2 ng/mL in Group 1 and Group 2, respectively. Box and whisker plot of the serum elabela level of both groups are depicted in Figure 2.

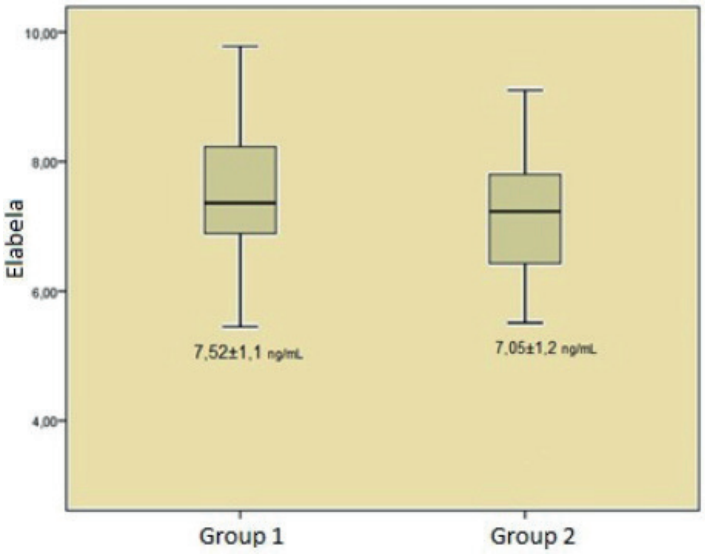

Figure 2. Box and whisker plot demonstrating serum elabela levels in Group 1 and 2.

Discussion

The diagnosis of ectopic pregnancy still presents several difficulties. Therefore, 1st trimester is the leading cause of maternal deaths. Early diagnosis and treatment are the key points of the disease. Elabela hormone is produced from trophoblasts in early pregnancy and plays a role in placenta formation throughout pregnancy [18]. A very recent study by Zhou and colleagues lent support to these observation [9]. The authors found that in women with late-onset preeclampsia, serum elabela levels were lower compared with gestational-age-matched healthy pregnant women. The study also revealed that placental mRNA and protein expression of elabela were significantly decreased in preeclamptic pregnancies. A combination of these accruing data with favorable effects of elabela supplementation in elabela-deficient mice on pre-eclampsia-like findings supports the crucial role of elabela in the pathogenesis of preeclampsia.

Serum elabela levels start to increase with conception. During second and third trimesters elabela reach significantly higher levels compared to the pre-pregnancy and first trimester levels [9]. Preeclampsia is grouped into two categories: early-onset and late-onset. Early-onset pre-eclampsia is defined as eclampsia occurring before the 34th gestation week [19]. Early-onset pre-eclampsia is considered primarily resulting from placentation defects. Studies investigating serum elabela levels have shown differences regarding early or late preeclampsia [20-22].

On the other hand, there appers some controversy in studies investigating serum elabela levels in the recent literature. First, serum elabela levels were found not different from normal pregnancies in patients with early-onset preeclapmsia [7, 8, 23]. Second, a recent study mentioned above by Zhou et al. [9] reported decreased serum elabela levels in late-onset preeclampsia, whereas subgroup analyses of the study by Panaitescu et al. [7] actually showed an increase in serum elabela levels in late-onset preeclamptic women. Lastly, some authors concluded that these differences may be due to differences in elabela essays that used in these different studies [24]. Thus, the conflict in findings of elabela change in hypertensive pregnancy disorders remains to be settled before drawing firm conclusions. Ectopic pregnancy is common and associated with significant morbidity and mortality. Currently, many cases of ectopic pregnancy are being missed, particularly when it presented in a subacute manner, and this substantially increases the risk of maternal death [25, 26]. In pregnant women who present with vaginal bleeding and/or abdominal pain, the current practice to diagnose ectopic pregnancy is combining serial measurement of serum beta-human chorionic gonadotropin (β-hCG) with transvaginal ultrasound. However, even this approach does not reach a 100% sensitivity; thus, indicating the requirement of novel biochemical markers for early detection of ectopic pregnancy. In recent years, accumulating evidence points that Elabela emerged as such a marker for preeclampsia. Since the pathogenesis of preeclampsia comprises aberrant placentation, uteroplacental ischemia, and endothelial dysfunction among others, it is not surprising that elabela levels are reduced in preeclamptic placentas. To our knowledge, elabela has not been studied outside the setting of preeclampsia in obstetrics practice. Since ectopic pregnancy is invariably associated with abnormal placentation, we thought that serum elabela levels may be abnormal and may provide as an aid for early detection for tubal pregnancy. The vast majority of ectopic pregnancies occur in fallopian tubes [27]. Fallopian tubes do not have a submucosal layer; thus, with tubal pregnancy, the fertilized ovum immediately burrows through the tubal epithelium. Quickly proliferating trophoblastic cells invade muscularis layer, in which zygote is located. Although we speculated that in tubal ectopic pregnancies, serum elabela levels might reduce, our results did not confirm this prediction. Women with ruptured ectopic pregnancy had slightly higher levels of serum elabela compared with control pregnancies. However, the difference did not reach statistical significance. Since ectopic pregnancy presents in the early gestational weeks, serum Elabela levels may not show a significant difference compared to the control group.

The negative finding in our study may have several plausible explanations. Firstly, lack of significant elabela change in patients with ectopic pregnancy compared with healthy pregnants might be in part due to the relatively small number of our patient cohort. Secondly, studies differ in their findings on serum elabela levels. Almost all available studies have shown that serum elabela levels were not different in women with early-onset preeclampsia. Since ectopic pregnancy results in perforation or hemorrhage early in the course of the pregnancy, serum elabela changes may resemble to the situation in early-onset preeclampsia, thus, the lack of any difference in serum elabela levels in ectopic pregnancies may be because of this. Lastly, serum elabela ELISA assays have not become standardized thorougly. Laboratory and clinical findings should be evaluated as a whole in the diagnosis of ectopic

pregnancy and possible delays should be prevented. Despite these shortcomings, this is the first study that attempted to evaluate serum elabela levels in ectopic pregnancy.

Conclusion

In contrast to findings reported for preeclampsia in the literature, the serum level of elabela was not different between women with ruptured ectopic pregnancy and women with uncomplicated pregnancies. Since ectopic pregnancy presents in the early gestational weeks, serum Elabela levels may not show a significant difference compared to the control group. Further studies with larger cohorts would shed more light on the role of elabela in ectopic pregnancy.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
This study received no financial support.

Ethical approval
This current study was carried out at Obstetrics and Gynecology Clinic of Fırat University between June 2018 and June 2019 after approval of the study protocol by the local ethics committee (IRB number: 2019/11-20).

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