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Research Article

Could ELABELA be a Protective Biomarker in Patients with Abnormal Uterine Bleeding?

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Abstract

Aim: Abnormal uterine bleeding (AUB) is a health problem characterized by various symptoms such as heavy and prolonged menstrual bleeding, affecting approximately 30% of female patients both physiologically and psychologically. The objective of this study was to assess serum Elabela (ELA) concentrations in women aged 18 and above diagnosed with functional AUB, and to compare these concentrations with those of healthy women.

Material and Method: This prospective case-control study was performed from August 18, 2022 to December 30, 2022. This was a cross-sectional study including 50 women who applied to the gynecology service of Malatya Turgut Özal Training and Research Hospital with complaints of AUB and 50 women without AUB who underwent gynecological examination. The presence of AUB in patients was determined based on clinical examination conducted by a gynecologist and medical records. Demographic and clinical characteristics were recorded. Serum ELA levels were determined by commercial ELISA kit.

Results: Serum ELA levels was significantly lower in patients with AUB (581.54±272.25 pg/mL) compared to the healthy group (744.55±300.31 pg/mL, p=0.005). In this study, ELA in patients with AUB showed 98% sensitivity and 80% specificity with a cut off value of 411.41 pg/mL (area under the curve [AUC], 68.1%; p=0.002).

Conclusion: Serum ELA levels in patients with AUB were significantly lower than in healthy women. These results show that ELA is a good predictor of the pathophysiological process of AUB.

Keywords: Elabela, abnormal uterine bleeding, PALM-COEIN

INTRODUCTION

The abnormal uterine bleeding (AUB), defined as bleeding exceeding 80 mL per menstrual cycle, affects approximately 3-30% of women worldwide, and up to 50% of women during the perimenopausal period (1,2). Functional AUB can profoundly affect a woman's quality of life, leading to physical, emotional, and social constraints, impaired sexual function, decreased productivity throughout the day, financial constraints, and negative effects on fertility and reproduction (3-5).

The patterns of AUB, which can be influenced by various factors such as hormonal imbalances, systemic conditions, uterine fibroids, polyps, endometrial hyperplasia, endometrial cancer, pelvic infections, certain medications, iatrogenic causes and bleeding disorders, are determined based on criteria including duration, intensity, regularity, and frequency of bleeding (3,6). The International Federation of Gynecology and Obstetrics (FIGO) has classified AUB as either structural causes (polyp, adenomyosis, leiomyoma, malignancy and hyperplasia [PALM]) or nonstructural causes (coagulopathy, ovulatory dysfunction, endometrial, iatrogenic, not otherwise classified [COEIN]) (7). The treatment of AUB can vary depending on factors such as the causes of bleeding, severity of symptoms, the patient's medical history, and preferences. It may involve medical treatments such as hormonal therapy, nonsteroidal anti-inflammatory drugs, levonorgestrel intrauterine devices or surgical treatment options such as endometrial ablation, myomectomy, or hysterectomy, based on the diagnosis (8,9). In the patient population presenting with AUB complaints, endometrial sampling is the gold

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standard for the most accurate detection of premalignant and malignant lesions. However, it is known that other diagnostic methods such as blood tests and ultrasound are insufficient (10).

The endogenous hormonal peptide known as elabela (ELA), also referred to as the G protein-coupled Apelin receptor agonist peptide (APLNR), consists of 32 amino acids (11). The expression of apelin/ELA-APJ is limited to several tissues including lung, prostate, adipose tissue, placenta, nervous system gastrointestinal tract, and vascular endothelium (11,12). Despite the research in the literature regarding ELA's involvement in embryonic development, bone homeostasis, angiogenesis, regulation of vascular and cardiac functions, anti-hypertensive effects, anti-renal fibrosis, prevention of kidney remodeling, and regulation of water homeostasis, its specific function in diverse pathophysiological events remains unknown (13). In recent times, the role of ELA in thrombosis has begun to attract attention. Moreover ELA may promote platelet aggregation, which plays a role in the formation of blood clots (14). Based on this information, we aimed to compare the serum ELA concentrations of women with AUB evaluated in accordance with the PALM-COEIN diagnostic criteria with those of healthy women without complications.

MATERIAL AND METHOD

The Research Methodology and Characteristics of the Study Participants

This study is a prospective case-control study including patients who presented with complaints of AUB to the Department of Obstetrics and Gynecology at Malatya Turgut Özal University Training and Research Hospital between August-December 2022.

All patients were investigated for the cause of bleeding according to the International Federation of Gynecology and Obstetrics PALM-COEIN classification through history, systemic examination, gynecological examination, and laboratory findings. Fifty women aged 18-49, diagnosed with menorrhagia, were included in the study after excluding identifiable pelvic pathology, endocrine diseases, or pregnancy, and meeting all specified exclusion criteria. The control set consisted of fifty healthy female volunteers, age- and body mass index-matched, who had regular menstrual cycles transpiring every 27-32 days. Power analysis was performed to determine the sample number (G Power 3.1.9.4).

Pregnant or lactating mothers, individuals with a body mass index of <18 kg/m² or >30 kg/m², those with polycystic ovary syndrome (PCOS), endocrine disorders, chronic systemic conditions, ectopic pregnancy, gestational trophoblastic disease, coagulopathy, or hematologic diseases, as well as those with benign or malignant ovarian, uterine, or vulvovaginal lesions, postmenopausal or hysterectomized women, users of medication, tobacco, alcohol, or drugs, and patients with intrauterine devices (IUDs) were not included in the study.

Biochemical Analysis

On the second or third day of menstruation, following an overnight period of not eating, blood samples were obtained from the arm veins of individuals in both the patient and control groups, using gel separator and anticoagulant tubes, between 08:00 and 09:00 A.M. The tubes were left to clot for 20–30 minutes. Once clotting occurred, tubes for collecting serum samples were spun at 1800 revolutions per minute (RPM) for 10 minutes at room temperature using a centrifuge.

A part of blood samples was used biochemical analysis platelet, mean corpuscular volume (MCV), hematocrit (%), hemoglobin, albumin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), C-reactive protein (CRP), iron, iron binding capacity, activated partial thromboplastin time (APTT), international normalized ratio (INR), and hormone analysis follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH). The analysis utilized a biochemistry device (Abbott Architect, USA) and a hormone device (Roche Diagnostics Cobas, Japan). ELA levels were determined by the commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bioassay Technology Corp., Cat. No: E7331Hu, China) following the guidelines provided by the manufacturer.

Statistical Analysis

The analyses of the data included in the study were conducted using SPSS (Statistical Program in Social Sciences) 25. The data's adherence to a normal distribution was evaluated through the Kolmogorov-Smirnov Test. Descriptive statistics such as mean, standard deviation, frequency, and percentage were employed. A significance level (p) of 0.05 was chosen for comparison tests. Since the variables followed a normal distribution ($p>0.05$), parametric test methods were employed for analysis. Comparisons in independent binary groups were conducted using the independent t-test. Receiver operating characteristic curve (ROC) analysis was applied to determine the cut-off point for a measurement value, and indices were calculated accordingly.

RESULTS

The study sample consisted of one hundred individuals in total, with fifty patients presenting AUB and fifty healthy controls. There were no statistically significant variations identified between the groups concerning demographic variables such as age and BMI ($p>0.05$) (Table 1).

According to the variables albumin, LDL, HDL, CRP, LH, APTT and INR, no statistically significant variations identified between the AUB and control groups ($p>0.05$). Significant statistical differences were noted between the AUB and healty control groups in terms of total cholesterol, iron, iron binding, hemoglobin, platelets, MCV, hematocrit, TSH, and FSH variables ($p<0.05$) (Table 2).

Table 1. Comparison of gender and BMI distributions between disease groups

Variable	Groups	Mean±SD	t	p values
Age (years)	AUB	38.09±5.79	0.840	0.404
	Control	31.59±6.43		
BMI (kg/m²)	AUB	26.37±2.8	0.836	0.406
	Control	24.16±2.92		

p value: statistical significance, sd: standart deviation, t: independent t test; *p<0.05: there is a statistical difference between the groups

Table 2. Comparison of laboratory biomarker values according to disease

Variables	Groups	Mean±SD	t	p values
Platelet (10³/µL)	AUB	330.79±77.84	**4.092**	**0.001***
	Control	270.43±65.26		
MCV (fL)	AUB	79.6±6.99	**-3.014**	**0.003***
	Control	83.3±5.2		
Hematocrit %	AUB	36.72±2.88	**-4.482**	**0.001***
	Control	39.3±3.03		
Total Cholesterol (mg/dL)	AUB	180.35±33.75	**2.357**	**0.020***
	Control	167.19±31.47		
LDL (mg/dL)	AUB	110.4±29.13	1.756	0.082
	Control	99.22±25.34		
HDL (mg/dL)	AUB	51.21±12.78	-0.105	0.917
	Control	51.23±10.98		
Iron (µg/dL)	AUB	48.6±28.97	**-4.412**	**0.001***
	Control	79.07±33.77		
Iron Binding Capacity (µg/dL)	AUB	341.83±71.31	**5.445**	**0.001***
	Control	262.08±58.93		
CRP (mg/dL)	AUB	0.42±0.79	1.742	0.085
	Control	0.17±0.27		
Albumin (g/dL)	AUB	4.28±0.37	-1.34	0.183
	Control	4.39±0.37		
Hemoglobin (g/dL)	AUB	11.92±1.28	**-5.139**	**0.001***
	Control	13.42±1.47		
FSH (mU/mL)	AUB	10.34±9.39	**3.863**	**0.001***
	Control	5.37±2.72		
TSH (mU/L)	AUB	2.16±0.91	**2.207**	**0.030***
	Control	1.8±0.75		
LH (mU/mL)	AUB	6.54±4.79	0.261	0.794
	Control	6.6±2.87		
APTT (s)	AUB	25.62±2.37	0.579	0.564
	Control	25.28±1.99		
INR	AUB	0.98±0.05	-0.615	0.54

p value: statistical significance, sd: standart deviation, t: independent t test, *p<0.05: there is a statistical difference between the groups; note: bold values are statistically significant values (p<0.05)

Evaluation of serum ELA levels

There was a statistically significant difference detected between the groups in serum ELA levels (p<0.05) (Figure 1, Table 3).

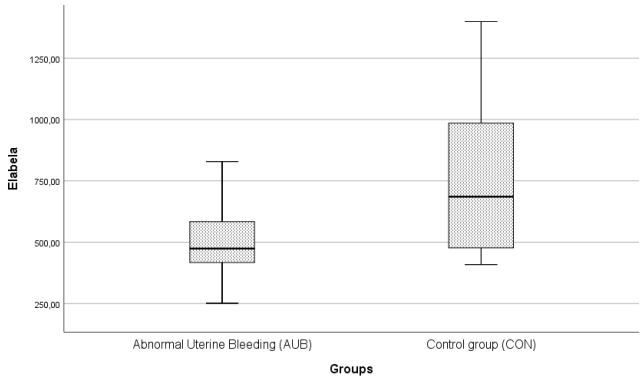

Figure 1. Box-plot of the distribution of elabela in groups

Table 3. The comparison of serum elabela levels in all study groups

Variables	Groups	Mean±SD	t	p value
ELA (pg/mL)	AUB	581.54±272.25	-2.844	0.005*
	Control	744.55±300.31		

p value: statistical significance, sd: standart deviation, t: independent t test, *p<0.05: there is a statistical difference between the groups; note: bold values are statistically significant values (p<0.05)

ROC curve analysis

ROC analysis was applied to determine the cut-off point for ELA measurement values. The analysis result is given in the table below. The areas under the curve calculated for ELA were found to be statistically significant (p<0.05, Figure 2). Serum ELA levels serve as a distinguishing factor in patients with AUB. When the ELA threshold was set at 411.41 pg/mL for AUB patients, the test showed 98% sensitivity, meaning it accurately identified 98% of those with AUB. Additionally, it displayed 80% specificity, indicating that it correctly ruled out AUB in 80% of cases where it wasn't present. (AUC=0.681, 95% CI=0.577–0.786, p=0.002) (Figure 2, Table 4).

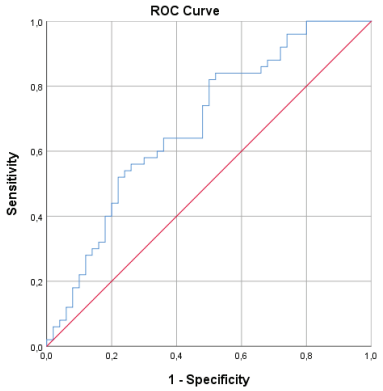

Figure 2. Receiver operating characteristic (ROC) curve analysis of the utility of ELA to predict AUB patients

Table 4. ROC analysis results of ELA levels

Test result variable(s)	Cut off	Sensitivity	Specificity	AUC	p value	95% C.I.for Exp (β)	
						Lower bound	Lower bound
ELA	411.41	0.980	0.800	0.681	**0.002***	0.577	0.786

AUC: area under the curve, C.I.: confidence interval

DISCUSSION

Abnormal uterine bleeding causes both physiological and psychological distress to female patients with various symptoms such as heavy and prolonged menstrual bleeding. This condition, affecting approximately ~30% of the female population worldwide, reduces the quality of life for women both physically and psychologically (1). In the patient population presenting with AUB complaints, endometrial sampling is the gold standard for the most accurate detection of premalignant and malignant lesions. However, it is known that other diagnostic methods such as blood tests and ultrasound are insufficient.

In this study, total cholesterol, iron binding, platelet, TSH, and FSH were found to be significantly high in AUB participants (p<0.05). However, iron, hemoglobin, MCV, and hematocrit levels were found to be significantly low in the AUB patient group compared to the healty group (p<0.05). Severe blood loss and menstrual periods lasting more than 7 days can lead to the depletion of iron stores in women, resulting in iron deficiency. Iron is crucial in numerous biological processes, encompassing DNA synthesis, cellular metabolism, and cell division. The decrease in iron levels affects hemoglobin levels. The reduction in hemoglobin levels disrupts and impairs RBC production, leading to the development of anemia (15,16). Karatoprak et al. reported an increased rate of iron loss and the development of anemia in women experiencing prolonged and heavy menstrual bleeding (17). FSH, produced by the anterior pituitary gland, plays a role in many physiological events such as gonadal sex hormone synthesis, menstruation, and follicular development. To compensate for the decrease in ovarian function before menopause, FSH increases before the decrease in estrogen (18). The enzyme 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) serves as the key regulatory enzyme in the biosynthesis of cholesterol. Guo et al. demonstrated that, during the premenopausal and perimenopausal periods, FSH significantly increases cholesterol levels progressively by regulating HMGCR (19). Our study results are consistent with the literature.

Additionally, our findings revealed that ELA expression was significantly lower in patients with AUB compared to the control group (p<0.05). Based on the results obtained, ELA could be a novel diagnostic biomarker for AUB. AUB is associated with molecular and cellular processes

such as decreased endometrial vasoconstriction, impaired endometrial angiogenesis, apoptosis, increased unpredictable vascular fragility through loss of integrity of endothelial, epithelial and stromal support structures and increased vascular fragility, defective hemostatic processes, cell proliferation, inflammation and repair mechanisms (2,10,15). Active peptides in the human body are integral to the maintenance of health and proper functioning of various physiological systems. Their dysregulation or imbalance can contribute to the development of various diseases and disorders (20).

Elabela peptide expression was found in humans in the prostate, kidney, renal distal collecting tubes, lungs, and veins, cardiovascular system, retina, ovary, testis, skin, stomach, placenta and embryonic stem cells (21). In recent studies, the ELA-apelin/APJ system has been found to be associated with many thrombotic diseases such as atherosclerosis, myocardial infarction, cerebral infarction, acute coronary syndrome, stroke and cancer (14,22). All these studies show that ELA/APJ signaling and regulating actions play a role in many physiological processes such as blood pressure control, cardiac and vascular modulation, hypertrophy, inflammation, apoptosis, angiogenesis, cell motility, cell proliferation and migration (23). It has been reported that the expression of Apelin/APJ plays a significant role in angiogenesis, metastasis, and cell proliferation, showing a significant increase in ovarian cancer (24). In another study, it was determined that Apelin and APJ receptor were expressed in obese individuals and women with presenting only ≥12 follicles per ovary and polycystic ovary syndrome (PCOS) (25).

Chen et al. reported that ELA, a hormone peptide belonging to the adipokine group and a component of the apelinergic system, activates the pannexin1 (PANX1)-P2X7 signaling pathway, inducing platelet aggregation and thrombosis (14). In an interventional study conducted by Coquerel et al., to evaluate the effects of ELA and Apelin-13 on vascular and cardio-renal function in a rat model of septic shock, it was demonstrated that ELA had superior effects on fluid homeostasis and cardiovascular hemodynamic recovery (26).

According to the results obtained in this study, a decline in ELA peptide expression was detected among AUB patients as opposed to the control group. In addition when the cut-off value for ELA was determined as 411.41 pg/mL in AUB patients, the sensitivity was 98% and the specificity was 80% (AUC=0.681, 95% CI=0.577–0.786, p=0.002). In line with these results, serum ELA levels serve as a predictive factor in patients with AUB.

Control of menstrual blood loss in women is achieved by vasoconstriction, hemostasis and re-epithelialization mechanisms. Local vasoactive mediators, which play a role in regulating vascular tone and hemostasis also have an important role in determining the amount of menstrual bleeding. However, expression of local endometrial factors has been observed to be abnormal in patients with AUB (27). Kacar et al. showed that rat apelin levels increased at the end of pregnancy and that apelin may be an endogenous peptide that triggers uterine contractions at birth (28). Apelin has been found to exert an inhibitory effect on spontaneous and oxytocin-induced contractions in human myometrium obtained during cesarean section (29,30).

The low serum ELA concentration in AUB patients may be explained by inadequate/slow hemostatic thrombus formation or insufficient vasoconstriction, leading to inadequate hemostasis. The significant decrease in serum ELA levels in AUB patients, along with the detection of high sensitivity (98%) and specificity (80%) at a cutoff value of 411.41 pg/mL, indicates that the ELA/APJ system plays an active role in AUB.

CONCLUSION

There was a significant decrease in serum ELA levels among patients with AUB when compared to healthy women. These results show that ELA is a good predictor of the pathophysiological process of AUB.

Limitations of the Study

Among the limitations of our study are its design as a single-center study and the relatively small sample size of the research population. These limitations may lead to the oversight of different factors that could contribute to the occurrence of AUB. More comprehensive prospective studies (detection in cervicovaginal fluids) could be useful in determining the predictive and therapeutic value of the ELA/APJ system in women with AUB.

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Conflict of interest: *The authors have no conflicts of interest to declare.*

Ethical approval: *The research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. The study was approved by the Ethics Committee of Malatya Turgut Özal University, Türkiye (Date: 18 Aug 2022, Issue: 2022/37). Written informed consent was obtained from each patient.*

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