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Analysis of the immunohistochemical and genetic expression pattern of kisspeptin in endometrial polyps

Analýza imunohistochemického a genetického vzorce exprese kisspeptinu v endometriálních polypech

Ş. İ. Paltacı[1], Ö. K. Günday[1], M. Ö. Erdoğan[2], F. Fırat[3], G. Ş. Yalçın[4], N. Akçalı[2]

[1] Department of Obstetrics and Gynecology, Faculty of Medicine, Afyonkarahisar University of Health Sciences, Afyonkarahisar, Turkey
[2] Department of Genetics, Faculty of Medicine, Afyonkarahisar University of Health Sciences, Afyonkarahisar, Turkey
[3] Department of Histology and Embryology, Faculty of Medicine, Afyonkarahisar University of Health Sciences, Afyonkarahisar, Turkey
[4] Department of Pathology, Faculty of Medicine, Afyonkarahisar University of Health Sciences, Afyonkarahisar, Turkey

Summary: Objective: Endometrial polyp (EP) is a type of pathology that is quite common in clinical practice. Although its exact etiology is not fully known, there is evidence to support that it is sensitive to hormonal stimuli. We aimed to investigate the relationship between kisspeptin (KP) and EP by comparing the genetic (tissue-blood) and immunohistochemical (IHC) expression of KP in EP lesions in patients with normal endometrial findings. **Materials and methods:** A prospective case-control study of 50 patients with EP (N = 25) and normal endometrial findings (N = 25) on biopsy and/or excision material was performed. Blood and biopsy samples obtained from all patients were stored at –80 °C. KP gene expression levels were determined from paraffin blocks, and peripheral venous blood samples obtained from biopsy specimens and IHC-H-score analysis were performed from paraffin blocks. EP and matched controls were compared for KP. **Results:** After IHC, the KP H-score of the control group was higher than the EP group, and this difference was statistically significant; H-score: control: 5 (++; 1–15); polyp: 1 (+; 0–12) ($P < 0.05$). Although KP expression in both tissue and blood was higher in the control group than in the EP group, this difference was not statistically significant ($P > 0.05$). No significant correlation was found between IHC H-score and KP expression levels in tissue and blood. According to the ROC analysis, the tissue and blood KP expression cut-off value and area under the curve (AUC) predicting the likelihood of developing EP were not significant (tissue KP: 1.04, AUC: 0.570, P = 0.388, sensitivity 56%, specificity 60%, Blood KP: 1.06, AUC: 0.569, P = 0.401, sensitivity 80%, specificity 40%). **Conclusions:** Decreased KP expression level in EP lesions may predict the diagnosis of EP, and in the future, KP may have therapeutic potential for benign gynecological pathologies such as polyps.

Key words: endometrium – endometrial polyps – kisspeptin – reverse-transcription PCR (RT-PCR) – immunohistochemistry

Souhrn: Cíl: Endometriální polypy (EP) představují patologii, která je v klinické praxi běžná. Ačkoli jejich přesná etiologie není úplně známá, existují důkazy o tom, že jsou citlivé vůči hormonální stimulaci. Naším cílem bylo prozkoumat vztah mezi kisspeptinem (KP) a EP pomocí srovnání míry genetické exprese (tkáň–krev) a imunohistochemické (IHC) exprese KP v lézích EP u pacientek s normálním endometriálním nálezem. **Materiál a metody:** Byla provedena retrospektivní případová studie u 50 pacientek s EP (n = 25) a normálním endometriálním nálezem (n = 25) z biopsie a/nebo materiálu z excize. Krev a vzorky z biopsie od všech pacientek byly uchovávány při –80 °C. Exprese genů KP byla stanovena v parafinových blocích a vzorcích periferní žilní krve získaných z bioptických vzorků a analýza skóre IHC-H byla provedena na parafinových blocích. EP a příslušné kontroly byly srovnány z hlediska KP. **Výsledky:** Po IHC bylo KP-H skóre v kontrolní skupině vyšší než ve skupině s EP a tento rozdíl byl statisticky významný; H skóre: kontroly: 5 (++; 1–15); polypy: 1 (+; 0–12) ($p < 0,05$). Ačkoli byla exprese KP jak v tkáni, tak v krvi vyšší v kontrolní skupině oproti skupině s EP, tento rozdíl nebyl statisticky významný ($p > 0,05$). V krvi ani tkáni nebyla zjištěna významná korelace mezi IHC-H skóre a expresí KP. Podle analýzy ROC cut-off hodnoty exprese KP v tkáni a krvi a plocha pod křivkou (AUC), která predikuje pravděpodobnost vzniku EP, nebyly významné (KP v tkáni: 1,04; AUC: 0,570; p = 0,388; senzitivita 56 %, specificita 60 % / KP v krvi: 1,06; AUC: 0,569; p = 0,401; senzitivita 80 %, specificita 40 %). **Závěry:** Snížená míra exprese KP v lézích EP může predikovat diagnózu EP a v budoucnu může mít KP při benigních gynekologických patologiích, jako jsou polypy, terapeutický potenciál.

Klíčová slova: endometrium – endometriální polypy – kisspeptin – PCR s reverzní transkripcí (RT-PCR) – imunohistochemie

Introduction

Endometrial polyp (EP) is a type of pathology that is quite common in clinical practice and is seen in all age groups, but is more common in women of reproductive age [1]. It is caused by hyperplastic overgrowth of endometrial glands and stroma around a vascular core, and although it is mostly benign, it needs to be differentiated from other uterine pathologies such as endometrial hyperplasia, leiomyoma, sarcoma, and carcinoma [2]. It is known that its prevalence varies between 7.8% and 34.9% depending on the population studied [1]. EPs can be single or multiple, stalked or sessile, and their sizes can vary from a few millimeters to centimeters [3]. It is one of the most common causes of menstrual irregularity and may cause postmenopausal bleeding and infertility [4]. Although its exact etiology is not fully known, there is evidence to support that it is sensitive to hormonal stimuli. For example, since EP is not reported before menarche, the effect of estrogenic stimulation is important [5], and it has been shown that the incidence of EP increases in postmenopausal women receiving hormone replacement therapy [5]. Additionally, compared to a normal endometrium, estrogen receptor concentration was found to be high in EP glandular cells, while progesterone receptor expression was found to be lower [6]. Although the first proposed mechanism is sensitivity to steroid hormones, other mechanisms such as steroid gene mutations [7], monoclonal endometrial hyperplasia [8], localized aromatase hyperactivity [9], high BCL-2 expression [10], and inflammation [11] have also been proposed in studies. Risk factors in the development of EP appear to be related to these mechanisms: obesity, age, tamoxifen use, and hypertension [12]. Polypectomy in patients with premenopausal symptomatic EP provides both treatment of symptoms and detection of malignancy, if any. In postmenopausal patients, it is recommended to remove all EPs, regardless of symptoms [2,13]. Hysteroscopic polypectomy is an effective treatment with low recurrence rates in patients with few EPs [14].

Kisspeptins (KPs) are a series of peptides of 54, 14, 13, and 10 amino acids produced by proteolysis of a 145 amino acid precursor protein encoded by the *KISS1* gene on chromosome 1. Nowadays, it is known that KP proteins, which carry common RF-amide (Arg-Phe-NH2) sequences in their C terminus, show agonist effects by binding to the GP54 receptor (GPR54) [15]. It has been observed that GPR54 is expressed at high rates in the placenta, pituitary, pancreas, and medulla spinalis, and it has been predicted that KP may have both endocrine and paracrine-autocrine functions [15]. These peptides have effects in regulating the release of GnRH, and stimulating the release of FSH and LH. Considering the role of FSH and LH in sex steroid secretion, which regulates cyclic endometrial changes, KPs are thought to play an important but indirect role in endometrial function [16]. Cejudo Roman et al. found that the neurokinin B/NK3R and KP/KISS1R systems are expressed in epithelial cells lining the lumen of the uterus and oviduct, suggesting that KP may have a peripheral role in the female genital tract [17]. It was previously known that epithelial cells in the endometrium and oviduct produce GnRH [18]. Therefore, it seems possible that KP regulates epithelial functions by participating in the peripheral regulation of GnRH secretion [17]. In a later study, Baba et al. reported that KP expression increased during the decidualization period, providing evidence that KP has effects on endometrial physiology [19]. In this study, they showed that KP secretion from endometrial glandular and stromal cells was affected by sex steroids (estradiol and progesterone), but GPR54 expression was found only in endometrial glandular cells and was not affected by sex steroids. Additionally, they did not monitor KP and KISS1R in the menopausal endometrium [19]. In their study in mice, Leon et al. reported that approximately 75% of endometrial gland formation (adenogenesis) is regulated by peripheral KP signaling and that extrahypothalamic KP is important for endometrial function, although the exact site of central release is unknown [20]. KPs have also been identified as regulators of matrix metalloproteinases (MMPs) at both the transcriptional and protein levels during their functions, including tumor metastasis suppression, placentation, and activation of the GnRH cascade, and this effect may be important for their peripheral effects in the uterus [21.

Since KP was first identified as a peptide derived from the metastasis suppressor gene [22], it was most frequently studied in malignant diseases [23]. There are fewer studies on benign uterine pathologies. For example, KISS1 protein expression was found to be higher in adenomyotic uteruses [24]. In a different study, serum KP and estradiol levels were found to be high in patients with EP [25]. The fact that EPs are estrogen sensitive, as in most benign uterine pathologies [5], and that KP causes systemic estrogenic effects by increasing local estrogenic activity in the hypothalamus may indicate its contribution to the development of EP [26]. There is also other important evidence for the peripheral effects of KP via estrogen. KP increases sensitivity to estrogen by increasing the expression of estrogen receptors in human granulosa cells [27]. Interestingly, in patients with polycystic ovary syndrome, serum KP levels were found to be higher and showed a positive correlation with estrogen levels [28]. Based on these findings, we planned to investigate the role of KPs, which are thought to be involved in the development of many cancers, in the etiopathogenesis of EP with malignant potential by simultaneously investigating their expression in tissue and peripheral blood by RT-PCR and immunohistochemical (IHC) staining.

Materials and methodology

This case-control study included 50 female patients aged 18–50 years between April 2021 and April 2022 who were admitted to a tertiary care hospital for menometrorrhagia and underwent diagnostic and therapeutic hysteroscopy-guided biopsy and/or excision, or endometrial biopsy, or dilatation-curettage (D&C). Pathology evaluation revealed normal endometrial findings (N = 25) and EP (N = 25). The study was approved by the University of Health Sciences Clinical Research Medical Ethics Committee (number: 2020/14; 547). The procedures complied with the terms of the Declaration of Helsinki. Written informed consent was obtained from all patients. Exclusion criteria were pregnancy, hormonal and/or oral contraceptive use for abnormal menstrual bleeding, malignancy, breastfeeding, pelvic infection, refusal to participate, and endocrine pathologies.

After the gynecological examination, ultrasonography examinations of all patients were performed using a vaginal probe by a single clinician in the dorsal lithotomy position (Logiq P5, GE Healthcare, Boston, Massachusetts, USA). The examination was performed regardless of the menstrual phase and with an empty bladder. Some of the patients with suspected EP underwent saline infusion sonography, and all patients with EP were offered hysteroscopy-guided excision, and consent for anesthesia and the procedure was obtained. Operative hysteroscopy (Karl Storz GmbH 26 Fr monopolar operative hysteroscopy set, Tuttlingen, Germany) was performed under anesthesia and operating room conditions. Cavity distension was achieved with a 5% mannitol solution. Flow pressure was set to a maximum of 120 mmHg. The endocervical canal, endometrium, space-occupying pathologies in the uterine wall, and bilateral tubal ostia were visualized. Soft formations with a smooth surface and a stalk or broad base covered with endometrium were defined as EP and excised using a resectoscope. Biopsies were taken from suspicious areas. In patients with no resectable pathology, curettage was performed with the appropriate Karman cannulas numbered 4, 5, and 6 and with the help of a Karman aspirator. In patients whose cervical os was not sufficiently open, some dilation was made using Hegar dilators, and the cannula was allowed to pass through the os (D&C).

The collected materials were sent to the pathology clinic in a 10% formaldehyde solution and evaluated by a single pathologist; routine hematoxylin-eosin staining was performed on all samples. Tissue KP gene and IHC expression analyses were applied to paraffin blocks whose pathology examination was completed and met the study criteria.

RNA isolation from peripheral blood

Peripheral venous whole blood was collected from all participants included in the study into two 2 mL EDTA tubes and processed for leukocyte isolation on the same day. RNA isolation from the obtained mononuclear cells was performed using a kit (QIAamp RNA Blood Mini Kit-Qiagen) with an RNA isolation feature from blood cells. Genomic RNA was obtained using the QIAamp RNA Blood Mini Kit procedure and stored at –80 °C, and the isolated RNAs were used to analyze mRNA expression levels.

RNA isolation from paraffin blocks

For the isolation of relevant RNA from paraffin-embedded tissue samples, genomic RNA was obtained using the Qiagen RNeasy FFPE Kit procedure and stored at –80 °C. The isolated RNAs were used to analyze mRNA expression levels.

Obtaining cDNA from RNA

Qiagen RT2 First Strand Kit was used to obtain cDNA from RNAs obtained from blood and tissue samples. The same amount of RNA was used for reverse transcription of each sample to be analyzed (an amount of 1–10 ng of total RNA per qPCR reaction is recommended). High-quality, nuclease-free water was preferred for the processes. The reactions were kept in a –20 °C freezer during storage.

Measurement of mRNA levels of the KP gene by real-time PCR

Following cDNA synthesis, the quantitative Real-Time PCR method was used to determine the expression of the KP gene (Qiagen RT2 qPCR Primer Kit). The region of interest was amplified in the real-time PCR device using primer pairs specific to the relevant genes, and GAPDH mRNA levels were taken as a reference when comparing the expression level.

Preparation of tissues for histopathological analysis

For histopathologic examination, tissue samples were fixed in 10% formaldehyde, passed through graded alcohol series and xylene for paraffin blocking, and 5 µm sections were placed on poly-L-lysine slides. The sections taken were stained with the IHC staining procedure. Five µm thick sections from the study tissues were boiled in the microwave for 28 minutes in citrate buffer (pH 6.0) for antigen retrieval. After washing, 3% H_2O_2 was applied for 5 min; the sections were washed three times for 5 min each with phosphate-buffered saline (PBS) and kept in a blocking solution for one hour for protein blocking. Then, primary antibodies were incubated with anti-KISS-1 (1/100, sc-101246 Santacruz) antibodies overnight at +4 °C. Washed sections were incubated with anti-mouse biotin-streptavidin hydrogen peroxidase secondary antibody for 30 minutes each. It was stained with aminoethyl carbazole (AEC). After nuclei staining with Mayer's hematoxylin, they were covered with a water-based covering medium, and IHC evaluations were made under a light microscope.

Tab. 1. Comparison of parameters for groups 1 and 2.
Tab. 1. Porovnání parametrů pro skupiny 1 a 2.

Parameters	Group 1 endometrial polyps	Group 2 control	P level
Age (years)	37 (27–50)	40 (27–50)	0.586
Gravida (N)	3 (0–9)	3 (0–6)	0.896
Parity (N)	2 (0–5)	3 (0–5)	0.305

All values are presented as median (Min.–Max.)
Mann-Whitney U-Test for differences between kisspeptin and control groups.

Histopathological evaluation method

A semi-quantitative method was used to evaluate IHC, and the histological score (H-Score) was evaluated according to the distribution area and staining intensity of the stained cells. The mean proportion of stained cells was graded as 1 for < 1%, 2 for 1–25%, 3 for 26–50%, 4 for 51–75%, and 5 for > 75% of the stained area. Staining intensity was graded as follows: 0 – negative saining; 1 – poor staining; 2 – moderate staining; 3 – strong staining. The histological score for each sample was calculated as follows: H-score = Degree of stained cell area x Average staining intensity. A total score between 0–15 was calculated and graded as negative (–, score: 0), poor (+, score: 1–4), moderate (++, score: 5–8), or strong (+++, score: 9–15) [29].

Statistical analysis

The Kolmogorov-Smirnov test was used to determine whether the data of the cases showed normal distribution. Descriptive statistics were shown as mean ± standard deviation or median (Min.–Max.) for continuous numerical variables and as number of cases (N) and percentage (%) for categorical variables. The Mann-Whitney U-Test was used to examine the differences between independent variables, and the Chi-square test was used for categorical comparisons. The relationship between the studied parameters was evaluated by the Spearman correlation analysis. Cut-off values were determined by performing the ROC analysis for KP. Statistical analysis was performed using the Statistical Program for Social Sciences version 21.0 (SPSS, Chicago, IL, USA). The level of significance was $P \leq 0.05$ for all statistical tests.

Results

Fifty patients, 25 with EP (patient group) and 25 with normal endometrial findings (control group) were included in our study. When the age and pregnancy status of the patients were evaluated, the mean age (mean ± SD) was 38.82 ± 6.52 years, gravida median: 3 (0–9), and parity: 3 (0–5). There was no difference between the two groups in terms of age, gravida, and parity ($P > 0.05$) (Tab. 1). D&C was performed in 54% of the cases, and hysteroscopy was performed in 46%. Hysteroscopy and pathology diagnoses of 15 out of 23 patients who underwent hysteroscopy were compatible (EP: N = 10; other: N = 5). When the KP expression levels in tissue and blood were compared between groups, the tissue KP level was 1.02 (0–1.66) in the EP group and 1.20 (0–1.64) in the control group. Blood KP levels were 1.26 (0–1.78) in the EP group and 1.29 (0–1.86) in the control group. Although KP expression levels in both tissue and blood were higher in the control group than in the EP group, this difference was not statistically significant ($P > 0.05$) (Tab. 2).

Tab. 2. Comparison of polyp and control group.
Tab. 2. Srovnání polypu a kontrolní skupiny.

Parameters		Group 1 endometrial polyps (N = 25)	Group 2 control (N = 25)	P level
Tissue kisspeptin expression (ng/dL)[a]		1.02 (0–1.66)	1.20 (0–1.64)	0.387[b]
Blood kisspeptin expression (ng/dL)[a]		1.26 (0–1.78)	1.29 (0–1.86)	0.399[b]
Tissue kisspeptin gene expression	negative	10 (40%)	8 (32%)	0.556[c]
	positive	15 (60%)	17 (68%)	
Blood kisspeptin gene expression	negative	9 (36%)	5 (20%)	0.208[c]
	positive	16 (64%)	20 (80%)	
Histologic Score (H-Score)[a]		1 (0–12)*	**5 (1–15)****	**< 0.0001[b]**

[a] Median (Min.–Max.), median [interquartile range].
[b] Mann-Whitney U-Test.
[c] Pearson Chi-Square.
* weak immunostaining of kisspeptin in group 1.
** strong immunostaining of kisspeptin in group 2.

When a comparison was made between the groups according to the presence or absence of KP expression, the rate of KP positivity in tissue was 60% in the EP group, while this rate was 68% in the control group. The KP positivity rate in blood was 64% in the EP group and 80% in the control group. There was no statistically significant difference in both tissue and blood KP expression in the EP and control groups ($P > 0.05$). IHC H-score in the control group was 5 (moderate staining), and 1 (weak staining) in the EP group. The H-score of the control group was higher, and this difference was statistically significant ($P < 0.05$) (Tab. 2, Fig. 1, 2). There was no significant correlation between KP gene expressions in tissue and blood or between KP gene expression and age ($P > 0.05$). There was no correlation between IHC H-score and KP gene expression levels in tissue and blood ($P > 0.05$). ROC curve was drawn for KP gene expression levels in tissue and blood, and the cut-off value was determined. According to the ROC analysis, the cut-off value and AUC of tissue and blood KP predicting the likelihood of developing EP were not significant (Tissue KP expression: 1.04, AUC: 0.570, $P = 0.388$, sensitivity 56%, specificity 60%, Blood KP expression: 1.06, AUC: 0.569, $P = 0.401$, sensitivity 80%, specificity 40%) (Fig. 3, 4).

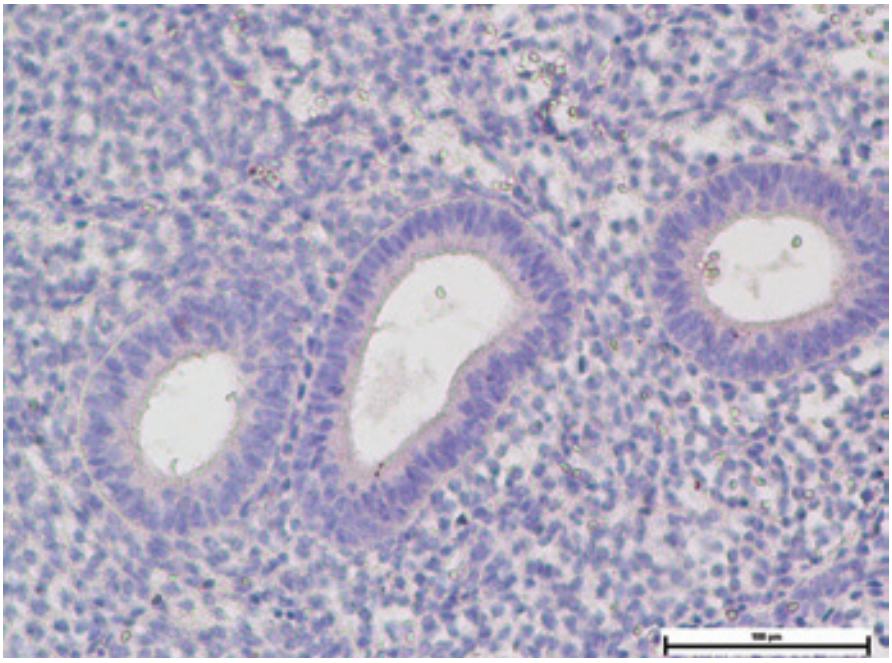

Fig. 1. Tissue kisspeptin immunohistochemical staining image in an endometrial polyp patient.
Obr. 1. Obraz imunohistochemického barvení tkáně kisspeptinem u pacientky s polypem endometria.

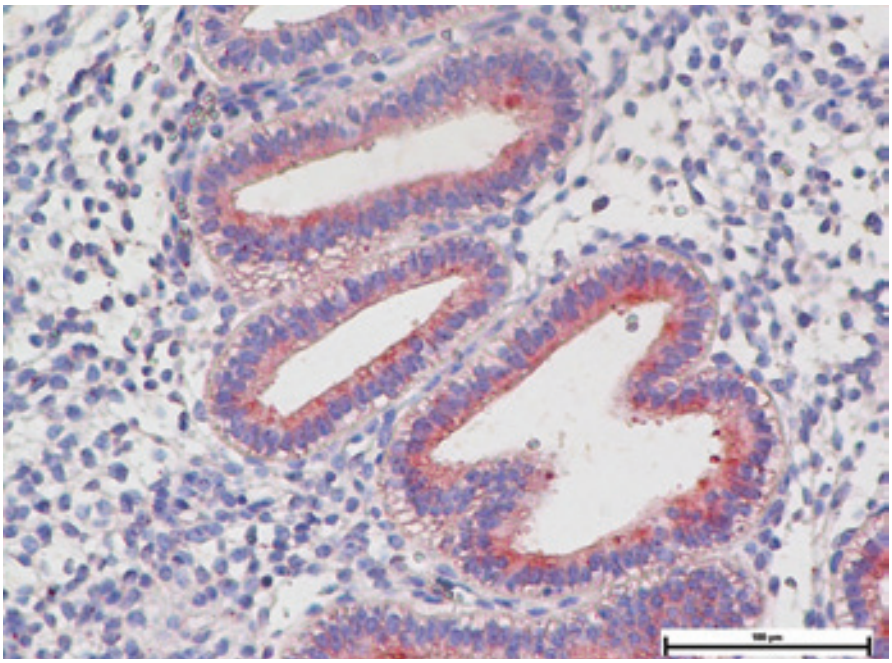

Fig. 2. Tissue kisspeptin immunohistochemical staining image in a control patient (the pink stained areas indicated by the red arrow are kisspeptin-dense areas).
Obr. 2. Snímek imunohistochemického barvení tkáňového kisspeptinu u kontrolní pacientky (růžově zbarvené oblasti označené červenou šipkou jsou oblasti s hustotou kisspeptinu).

Discussion

In the EP group, the IHC H-score of KP was poor and was found to be significantly lower than the control group with moderate staining ($P < 0.001$). KP gene expression was also lower in the EP group than in the control group, but the difference was not significant. We could not find a study in which KP expression in EP and endometrial tissue was evaluated simultaneously with both RT-PCR and IHC H-score. After the first discovery of KP as a metastasis suppressor in malignant melanoma [22] and its role in cell invasion through MMP regulation [30], it is seen that most of the studies have been conducted in malignant tumors [31]. In a study including both malignant endometrial cancer (EC) and benign endometrial lesions, IHC staining for KP was performed in endometrial tissues of 169 patients diagnosed with normal

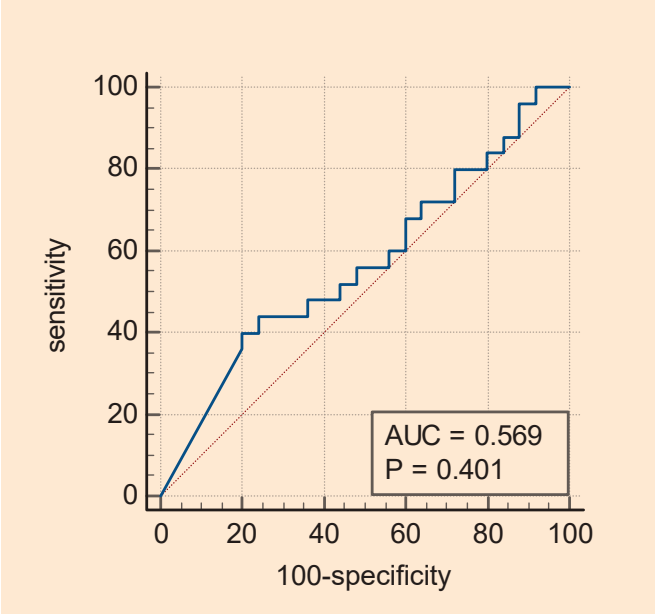

Fig. 3. Receiver operating characteristic (ROC) curve for blood kisspeptin for prediction of a polyp (AUC: 0.569, P = 0.401).
Obr. 3. Křivka ROC pro krevní kisspeptin pro predikci polypu (AUC: 0,569; p = 0,401).

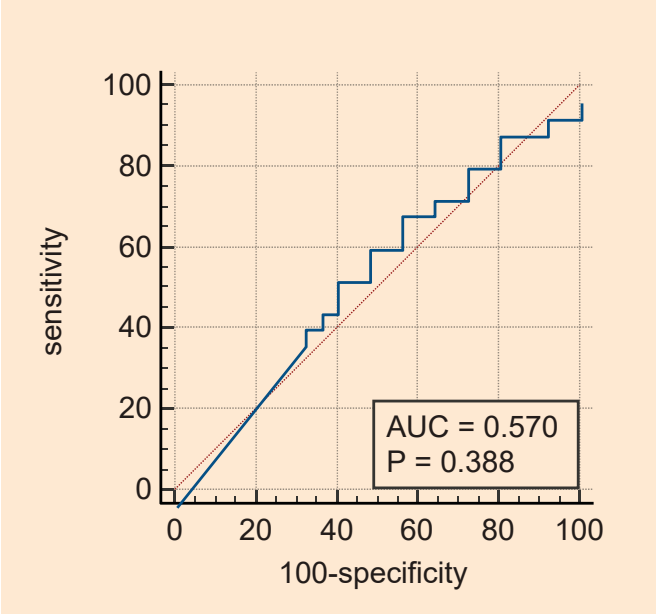

Fig. 4. Receiver operating characteristic (ROC) curve for tissue kisspeptin for prediction of a polyp (AUC: 0.570, P = 0.388).
Obr. 4. Křivka ROC pro tkáňový kisspeptin pro predikci polypu (AUC: 0,570; p = 0,388) .

endometrium (NE), EP, hyperplasia on EP background (EP-h), endometrial intraepithelial neoplasia in EP (EP-EIN), and EC [32]. All patients in the NE, EP, and EP-h groups showed weak staining with KP, while moderate to strong staining was observed in EP-EIN and EC groups. Contrary to our study, no difference was observed between a polyp and NE in terms of KP staining. In another study, an analysis of 92 EC cases revealed that overall survival improved in cancers with higher GPR54 expression and that GPR54 expression was associated with FIGO stage and deep myometrial invasion [33]. Low KISS1R protein and KISS1R mRNA expression were associated with invasion of the lymphovascular space and myometrium in EC patients, and exogenous KP administration limited metastasis [33]. Deficiency of KP and KISS1R in the endometrium may lead to altered invasion and angiogenesis of the endometrium as a result of abnormal expression of MMPs, vascular endothelial growth factor (VEGF), and other molecules. Two important processes in the pathogenesis of diseases associated with abnormal endometrial hyperplasia are the limitation on migration and invasion of endometrial cells and the suppression of angiogenesis [34]. The same situation may also be valid for EPs, and the fact that KP IHC staining was lower in EP tissues in our study including lower simultaneous tissue KP expression in the EP group, although not significant, it supports this hypothesis. In our study, there was no significant correlation between IHC KISS1 H-score and blood and tissue KISS1 expression. This may be because KISS1 protein production and tissue mRNA expression in the blood are not directly related and may be due to other hormone and protein interactions in the blood [35,36].

In uterine pathologies such as atypical endometrial hyperplasia and EP, patients often present with menstrual disorders, and most patients have unopposed estrogen exposure. Baba et al. reported that in atypical endometrial hyperplasia, endogenous metastin secretion may be decreased in endometrial secretory cells, and decreased metastin in EC may facilitate myometrial invasion and metastasis [19]. These diseases are a significant problem in young patients seeking uterine-sparing treatment. KP may have potential as a suitable medical therapeutic agent both in these patients [19] and in benign gynecological pathologies such as EP [19].

Endometriosis is one of the pathologies in which functional endometrial glands and stroma are located outside the uterine cavity and whose pathogenesis is related to increased invasion and migration ability of the endometrium. Abdelkareem et al. compared KISS1--GPR54 activity in eutopic endometrium (N = 35) in women with endometriosis and endometrium (N = 14) in women without endometriosis by IHC staining showing that KISS1 and GPR54 levels were decreased in eutopic endometrial stroma in the endometriosis group compared to the control group [37]. Most importantly, among ectopic lesion types, KISS1 levels were found to be decreased in deep infiltrative versus superficial en-

dometriotic implants. They concluded that lower levels of KISS1 resulted in more invasive disease and easier migration of endometrial cells into the peritoneal cavity through the tubes [37]. KP may have this effect by reducing the expression of MMPs that play a role in invasion [38]. Erdemoglu et al. showed the expression of epithelial and stromal MMP-2 and MMP-9 in EPs obtained from both premenopausal and postmenopausal women [11]. Considering the role of MMPs in EP pathogenesis and the KP-MMP relationship, according to the results of our study, decreased KP expression in EP may have participated in EP formation through MMP interaction.

VEGF is an important molecule that regulates angiogenesis and is important for the angiogenesis of spiral arteries and the restoration and hyperplasia of the endometrium during the normal menstrual cycle [39]. It has been reported that KP, in addition to its interaction with MMPs, suppresses VEGF expression [40], and the upregulation of angiogenesis in ectopic endometrium in endometriosis [41] supports this effect of KP. Furthermore, VEGF and transforming growth factor beta-1 were found to be significantly higher in EPs compared to normal endometrium [42], supporting the fact that KP deficiency in the endometrium may result in EP development through MMP and VEGF upregulation. In another study, KISS1 protein levels were measured by IHC staining in adenomyotic tissue samples (N = 29), eutopic endometrium (N = 29) from the same patients, and normal endometrium (N = 29) from patients without adenomyosis [24]. In this study, in contrast to the endometriosis study, KISS1 staining level was found to be significantly higher in adenomyotic tissues and ectopic tissue of adenomyotic patients than in eutopic endometrial tissue and patients with a normal endometrium. In a 2005 study, KISS1 and GPR54 mRNA expression was analyzed by RT-PCR in a total of 54 patients, including 32 patients with endometrial carcinoma, 10 patients with EIN, and 12 patients with a normal endometrium. KISS1 mRNA expression was found to be 37.5%, 80%, and 83.3% in EC, EIN, and normal endometrial tissues, respectively. KISS1 expression in EC was negatively correlated with myometrial invasion and lymph node metastasis ($P < 0.05$). The positivity rate of GPR54 mRNA in EC, EIN, and normal endometrium was found to be 78.1%, 70%, and 66.7%, respectively, without any significant statistical difference ($P > 0.05$). We concluded that the interaction of KISS1 and GPR54 may play a role in preventing EC invasion and metastasis. It has even been suggested that KP has the potential to be a new therapeutic agent that will inhibit metastasis in urogenital carcinomas [43]. However, studies are showing that KP expression changes in different directions in different organ cancers [23].

For the effect of KP on EPs, interactions such as MMP and VEGF, as well as interactions with estrogen, should be taken into consideration. While KP-10 initiates cell invasion in ERα-negative breast cancer, this effect is not observed in ERα-positive breast cancer cells, indicating that KP/KISS1R regulates cell invasion in an ERα-dependent manner [44]. Also, studies are showing the presence of high estrogen receptor expression in EPs [6], and that KP strengthens the local and systemic estrogenic effects in the hypothalamus [45]. In addition, the treatment of hormone-dependent diseases such as endometriosis, precocious puberty, and leiomyoma with KP antagonists has been recommended [46]. Based on this, KP may be considered as a treatment option in the future for EP, which is a benign uterine pathology that is estrogen-sensitive [5,6].

In our study, the KP gene expression level in the blood was lower in the EP group than in the control group, but the difference was not significant ($P > 0.05$). Yıldırım et al. [25] found the KP level in the blood to be significantly higher in the EP group (mean 1.84 ng/dL) than in the control group (mean 1.32 ng/dL) ($P = 0.008$). The reason for the different results in the studies may be the difference in the measurement techniques used, the presence of many endocrine and physiologic variables affecting the level of KP, and the interaction of KP with other hormones (insulin, leptin, prolactin, etc.). In addition, the fact that tissue and blood KP and KP receptor status are unknown in most studies and that IHC staining offers the only parametric measurement of KP in some studies seems to make it difficult to interpret the results.

The strengths of our study include the fact that KP expression was examined in both tissue and blood at the same time and that the level of KP in tissue was evaluated by the RT-PCR method in addition to the IHC H-score, allowing the evaluation of not only systemic effects but also autocrine-paracrine effects of KP and its prospective design. In the literature, most studies on KP have focused on the anti-metastatic function. However, there are knowledge gaps regarding how it regulates local endometrial function. We believe that our study makes an important contribution to this knowledge gap. However, our study has some limitations. The patient population is relatively small; the menstrual phase is not taken into account, and results from a single clinic may not be generalizable to the whole population. It is necessary to understand the complex interactions regarding the role of KP in endometrial pathologies and to clarify the actual peptide that plays a role in endometrial regulation through more comprehensive studies for each of the molecules in the KP family.

In conclusion, in our study, KP showed significantly less staining in EP lesions than in normal endometrial tissues. Our study contributes to our understanding of the role of KP in EP, one of the most common benign endometrial lesions. In addition, KP [33], which continues to be

investigated for therapeutic use, may be promising as a treatment method to prevent the development and recurrence of Eps.

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Authorship

ÖKG, SİP: concept and design
ÖKG, MÖE, FF, GŞY, NA: ccquisition, analysis, or interpretation of data
ÖKG, SİP: drafting of the manuscript
ÖKG, MÖE, FF, GŞY, NA: critical review of the manuscript for important intellectual content
ÖKG, MÖE, FF, GŞY, NA: supervision

ORCID authors

Ş. İ. Paltacı 0009-0006-1038-2075
Ö. K. Günday 0000-0002-9249-679X
M. Ö. Erdoğan 0000-0002-3434-8545
F. Fırat 0000-0003-0027-5138
G. Ş. Yalçın 0000-0002-1944-2955
N. Akçalı 0000-0001-6816-9687

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Assoc. Prof. Özlem Kayacık Günday, MD
Department of Obstetrics and Gynecology
Faculty of Medicine
Afyonkarahisar University of Health Sciences
Zafer Sağlık Külliyesi Dörtyol Mahallesi
2078 Sokak No:3
Afyonkarahisar, Turkey
kayacikozlem@yahoo.com.tr

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