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Original Article

Is there a relationship between serum kisspeptin levels and endometrial polyps in women with premenopausal status

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Abstract

Aim: Endometrial polyps are frequently associated with abnormal uterine bleeding. The kispeptin family is one of the peptides that play a role in reproductive functions and whose expression varies in various uterine pathologies. The aim of the study was to determine the relationship between serum Kisspeptin levels and endometrial polyps in women with premenopausal status.

Material and Methods: The blood was collected prior to endometrial sampling from women admitted to the hospital due to abnormal uterine bleeding. According to the pathology results, patients were identified as polyp group (n=38) (endometrial polyps) and control group (n=50) (normal endometrial findings). Kisspeptin-54 levels were determined by ELISA method from serum obtained from venous blood.

Results: There were no difference was found between the patients' age, body mass index, gravida, para, abortus and the number of living children were compared (p>0.05). There was no statistically significant difference between the groups in terms of follicle stimulating hormone, luteinizing hormone and thyroid stimulating hormone values (p>0.05). Plasma kisspeptin (1.84±0.93 ng/dL and 1.32±0.47 ng/dL, p= 0.008) and estradiol (90.34±13.02 pg/mL and 81.75±12.36 pg/mL, p=0.002) levels were significantly higher in the polyp group than in the control group. After the Receiver Operating Characteristic (ROC) analysis the area under the curve (AUC) was 1.26 (p= 0.08), (95% CI, 0.550-0.782). The sensitivity value was 0.684 (0.512-0.819), the specificity was 0.620 (0.471-0.750).

Conclusion: The serum Kisspeptin-54 and estradiol levels were found higher in patients with endometrial polyps.

Keywords: Abnormal uterine bleeding; endometrial polyps; kisspeptin.

INTRODUCTION

Endometrial polyps (EP) can be seen in all age periods, more frequently in women of reproductive age. These structures, which are formed by the localized hyperplasia of the endometrial gland and stroma surrounding a vascular center, are generally asymptomatic, but are one of the common causes of menstrual irregularities and postmenopausal bleeding, also associated with infertility-subfertility (1-3). Although molecular mechanisms such as monoclonal endometrial hyperplasia, localized aromatase hyperactivity, and steroid receptor gene mutations have been implicated in its development, there is no clear consensus on its occurrence (4-6).

Kisspeptins are a series of structurally linked peptides produced by proteolysis of a 145-amino acid precursor encoded by metastase supressor gene KISS 1 located on chromosome 1 (7). These peptide functions by activation of GPR54 that matches G protein and modulates the cell proliferation (8). The effects of Kisspeptin on endometrial physiology, which has a role in reproductive functions, were determined by increased its expression in desidualization period (9,10). Expression of kisspeptin protein was also investigated in different uterine pathologies. For example, the Kisspeptin protein KISS-1 expression was found to be higher in adenomyotic uterus cases (11). The role of Kisspeptin which has modulatory effects on cell proliferation, in the development of EP has not been

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investigated according to our current knowledge. Based on this background, in the current study, it was aimed to investigate the kisspeptin levels in peripheral venous blood in premenopausal women with EP in this study.

MATERIAL and METHODS

Our study included women of premenopausal period who presented to the gynecology outpatient clinics of the Department of Obstetrics and Gynecology, Hitit University, Faculty of Medicine, due to abnormal menstrual bleeding. The prospective cohort study was conducted between January 2018 and November 2018, in Corum, Turkey. Our study was approved by Hitit University Medical Faculty Clinical Research Ethics Committee in accordance with Helsinki Declaration (Approval date: 12/19/2017, Approval number: 2017-194). Informed consent was obtained from the volunteers who participated in the study.

The study included 863 women aged between 18 and 40 years, who were irregular in their last three menstruation. The inclusion and exclusion criteria were used for patient selection. İnclusion criteria were determined as; being in the age range of 18-40, irregular menstruation in the last 3 months, to accept the necessary medical interventions and biopsies, post-biopsy pathology results include endometrial polyp and normal endometrial findings determined. Exclusion criteria were determined as; having suspected or proven pregnancy, using hormone or oral contraceptives during the period of abnormal menstrual bleeding, having intrauterine devices, having endocrine pathologies and presence of urological diseases that may cause hematuria.

Having suspected or proven pregnancy, Presence of urological diseases that may cause hematuria, having Endocrine pathologies

Endometrial biopsy was planned for the remaining 217 women after the inclusion and exclusion criteria for 863 women. Those who refused the biopsy, those who refused to participate in the study, those who did not want to be included in the study, the biopsy material was inadequate for the diagnosis, the cervical smear test was pathological, and the pathology revealed a diagnosis other than polyp or normal endometrial findings, 88 patients remained (Figure 1). The pathological results of these patients were reported as EP (n = 38) and control group (n = 50) with benign physiological findings.

At the time of presentation, medical history and family history were taken. Physical examination, pelvic and vaginal examinations were performed. Body mass indexes (BMI) were calculated by calculating their height and weight. The patients were evaluated by transvaginal ultrasonography (Logiq P5, 2015, GE Healthcare, Milwaukee WI). Endometrial thickness, endometrial and myometrial echogenicity and adnexal areas were examined and recorded during ultrasonographic evaluation. When endometrial evaluation revealed space-occupying lesions in the uterine cavity, saline infusion sonography (SIS) and hysteroscopy (HS) were applied.

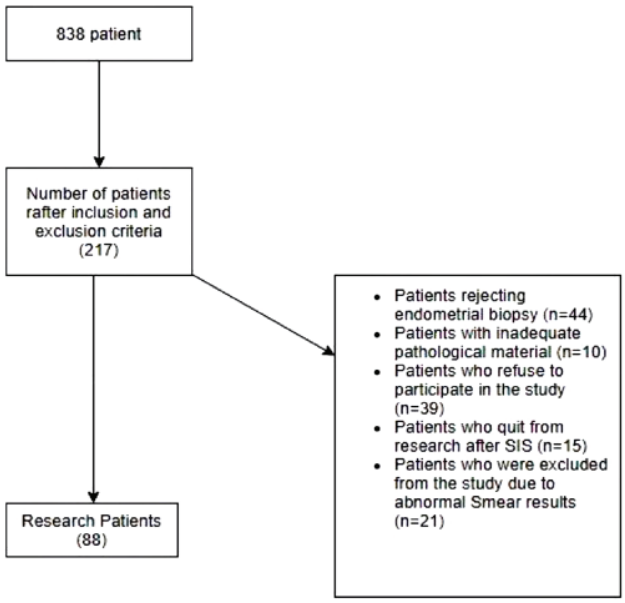

Figure 1. Flow chart of participant selection

Endometrial biopsies were planned in patients with abnormal uterine bleeding after the examination and sonographic evaluation. All biopsies were taken in the follicular phase between 7-16th days of menstrual cycle. Before the endometrial biopsies or hysteroscopic biopsies of the patients, routine blood tests were performed by venopuncture and 5 ml blood was separated. For the measurement of FSH, LH and E2 samples were allowed to clot at room temperature. Within 30 min after coagulation, the samples were centrifuged at 1000 x g for 20 minutes. The sera were analyzed by the electrochemiluminescence immunoassay (ECLIA) method using an auto-analyzer (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). All blood samples were centrifuged within 30 minutes after vein puncture. The obtained serum was frozen at −80° C till further analysis using a Kisspeptin -54 ELISA Kit (Rel Assay Diagnostistics, Kiss RLD 3123 ELISA Kit, Turkey). ELISA kit kit recognizes natural and recombinant Human KISS-54 with colorimetric methods. Detection limits were 0.0625-5 ng/dl.

Endometrial biopsies were performed with Karman vacuum aspiration. Hysteroscopic surgical procedures were performed in the operating room by operative hysteroscopy. In order to prevent bias, pathologists were not informed about the study groups. Pathological results were shared with patients and appropriate treatment was performed. Patients with EP were determined as study group, and patients with normal endometrial findings were determined as control group. Patients with endometrial pathologies different from the above mentioned diagnosis were excluded from the study.

Statistical Analysis

Data were analyzed using SPSS (Version 22.0, License; Hitit University). Descriptive statistics were presented as mean ± standard deviation and median (min-max)

according to data distribution; and nominal variables were presented as number of cases and percentage. The distribution of the data was analyzed with Shapiro-Wilk test. Data with normal distribution were compared with the Student's t-test and the data that did not comply with normal distribution were compared with the Mann Whitney-U test. In the estimation of benign endometrial pathologies, it was investigated statistically whether Kisspeptin levels were diagnostic and prognostic markers. In order to determine the discriminant power of the index (maximum sensitivity and selectivity) using ROC (Receiver Operating Characteristic) analysis method, the ROC graphs were drawn, the area under the curve (AUC) and the 95% confidence intervals of this area were calculated. In the analyzes, the significance of determining the risk group was taken as AUC> 0.500 (0.9-1: Excellent, 0.8-0.9: good, 0.7-0.8: moderate, 0.6-0.7: weak and 0.5-0.6: failed). Sensitivity, selectivity, positive predictive value, negative predictive value and positive likelihood values were calculated for these variables. Youden index was used to determine the best cut-off point in ROC analysis. P value <0.05 was considered significant.

RESULTS

According to the pathological diagnosis of the patients, the patients were divided into two main groups as EP and control group. Statistical analysis was performed according to the groups formed by the pathological results. Diagnosis of 24 (63.2%) of the patients with EP was made by vaginal ultrasonography. SIS was performed in 9 of these 24 patients and hysteroscopic polypectomy was performed in 2 patients. Cervical smear test was performed before biopsies of all groups. After cervical smear, inflammation was observed in 11 patients (12.5%) and atypical squamous cells of undetermined significance (ASCUS) were found in 2 (2.27%) patients. The remaining patients had normal smear tests (85.23%). When the demographic characteristics of the patients were compared, no statistical difference was found between the patients' age, body mass index, gravida, para, abortus and the number of living children. There was no statistical difference between the patients' biopsy days (Table 1).

There was no statistically significant difference between the groups when compared with follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH) values (p = 0.197, 0.402, 0.070). Estradiol levels were compared and a statistically significant difference was found (p=0.002). When the Kisspeptin levels of the groups were compared, a statistically significant difference was found and showed in the box-plot graph (p = 0.008) (Table 2). The kisspeptin levels of the women in the EP group were significantly higher than the women with physiological endometrial findings.

In the prediction of EP, the AUC value of Kisspeptin level was 0.666 (95% CI, 0.550-0.782) and the cut-off point was 1.26, and p value was determined as 0.08 (Figure 2, Table 3).

Table 1. Demographic Characteristics of Groups

	Endomerial Polyp (n=38) (Mean±Std) (Median. min-max)	Endomerial Polyp (n=50) (Mean±Std) (Median. min-max)	P value
Age (Year)	34.52±4.64 35.00 (24.00-42.00)	34.22±3.13 34.50 (25.00-39.00)	0.713[a]
BMI kg/m^2	22.52±1.83 22.00 (20.00-26.50)	22.59±1.83 22.00 (20.00-28.00)	0.928[b]
Gravida	2.31±1.04 2.00 (0-5.00)	2.36±0.85 2.00 (1-4.00)	0.740[b]
Para	2.01±0.93 2.00 (0-4.00)	2.06±0.79 2.00 (1.00-4.00)	0.971[b]
Live	1.92±0.88 2.00 (0-4.00)	2.06±0.79 2.00 (1.00-4.00)	0.772[b]
Abortus	0.34±0.58 0 (0-2)	2.06±0.79 2.00 (1.00-4.00)	0.745
Biopsy Day	9.86±2.29 10.00 (5.00-14.00)	11.22±2.41 11.50 (4.00-15.00)	0.090[a]

aStudent's T test, bMann-Whitney U test, BMI: Body Mass Index

Table 2. Biochemical Data of Groups

	Endomerial Polyp (n=38) (Mean±Std) (Median min-max)	Endomerial Polyp (n=50) (Mean±Std) (Median min-max)	P value
FSH (IU/L)	8.62±0.99 8.90 (5.87-10.33)	8.33±1.18 8.67 (5.64-11.31)	0.197[b]
LH (IU/L)	7.13±1.19 7.14 (4.36-9.31)	6.60±1.38 6.67 (4.14-9.77)	0.402a
E2 (pg/mL)	90.34±13.02 91.00 (56.33-16.67)	81.75±12.36 82.67 (65.21-115.36)	0.002[b]
TSH (IU/L)	1.30±0.41 1.34 (0.66-2.05)	6.60±1.38 6.67 (4.14-9.77)	0.070[a]
Kisspeptin (ng/mL)	1.30±0.41 1.34 (0.66-2.05)	6.60±1.38 6.67 (4.14-9.77)	0.008[b]

[a]Student's T test, [b]Mann-Whitney U test, FSH: Follicular Stimulating Hormone, LH: Luteinizing Hormone, E2: Ostradiole, TSH: Thyroid Stimulating Hormone

Table 3. Roc curve results

	Kisspeptin
AUC (95 % CI)	0.666 (0.550-0.782)
p	0.008
Cut-off	>1.2615
Sensitivity	0.684(0.512-0.819)
Specificity	0.620 (0.471-0.750)
PPV	0.577 (0.422-0.720)
NPV	0.488 (0.279-0.577)
LR+	1.800 (1.189-2.725)

AUC: Area Under the Curve, PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR+: Positive Likelihood Ratio

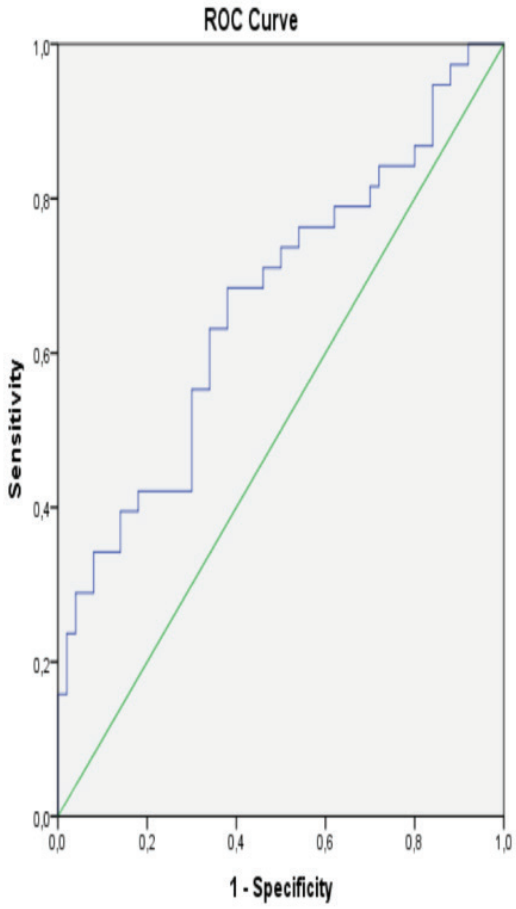

Figure 2. The ROC curve of Kisspeptin for predicting endometrial polyp group

DISCUSSION

Endometrial sampling plays an important role in the diagnosis of female benign disorders endometrial polyps (12). Hysteroscopic sampling is the gold standard diagnostic method (13). In our study, we used both metods and found that serum Kisspeptin levels were higher in patients with EP and this difference was significant when compared to the control cases. To evaluate the use of Kisspeptin as a biochemical marker to determine the development of polyps, we performed ROC analysis and found the predictive value low.

Based on the findings of recent researches focusing the role of Kisspeptin and its receptors on hypothalamus-hypophysis-gonad axis, it may be considered that, the protein may have a critical role in the development of gynecological pathologies including EP (14).

Studies investigating the etiopathogenesis of EP have found some evidence about the role of estrogen. For example; in immunohistochemical studies, EP are high estrogen receptor expression was shown (15). In addition, there are studies showing that EP are caused by apoptosis mechanism pathologies (16). The high estrogen levels in our study suggest that EP develop on this basis. Similarly, we found high kisspeptin levels in patients with EP.

It is also well documented that Kisspeptin leads to increased local estrogenic activity in hypothalamus as well as potentiate the systemmic estrogenic effects (17,18). Firstly, the relationship between kisspeptin and sex steroids was demonstrated in rat studies. In female rats, the expression of pituitary Kiss-1 mRNA was decreased after gonadectomy. Then, Kiss-1 mRNA expression was normalized after estradiol injection to gonadectomized rats (19). The effects of Kisspeptin on reproductive functions have been shown in human studies. For example; Hypogonadotropic hypogonadism was found in human and mice with kisspeptin gene mutation (20).

As increased estrogenic activity has been accepted the leading theory in the development of EP, higher Kisspeptin levels in women with EP, found in the current study may have a role of the occurence of estrogen-dependent gynecological pathologies. It has been observed in previous studies that increased serum kisspeptin concentrations are associated with significantly higher LH and basal estradiol production (21). Gonadotrophins under the influence of kisspeptin have been shown to manage oestradiol production by granulosa cells in the ovaries. In addition, estradiol can suppress the level of kisspeptin with negative feedback through the neuronal pathway (8).

Similar with our findings, Kisspeptin levels were found to be higher in patients with anovulatory cycle and in patients with polycystic ovary syndrome as well as positive correlation with estogene levels (22,23). Estrogen increases the LH secretion and the positive feedback relationship between LH and Kisspeptin can explain the higher Kisspeptin serum levels in women with polyps (24).

To our knowledge, the present study is the first one that investigated the association of kisspeptin with EP. The main limitations of our study are the low number of patients and not being combined with kisspeptin levels in the endometrial tissue. In our study, the increased serum Kisspeptin finding in women with EP needs to be demonstrated by tissue kisspeptin measurements and gene expression studies in polyp tissues.

CONCLUSION

In our study, serum Kisspeptin and estradiol levels were found to be higher in patients with EP. For clinical use, studies should be performed in larger series.

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