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ORIGINAL ARTICLE

Altered expression of kisspeptin in polycystic ovarian syndrome; can we rely on?

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ABSTRACT

Objective: Polycystic ovarian syndrome (PCOS) is a complex endocrinopathy that affects women in reproductive age. Although the pathogenesis is still unclear, the abnormal secretion of luteinizing hormone, follicular-stimulating hormone due to the alterations in hypothalamic–pituitary–gonadal (HPG) axis, is demonstrated to be the major cause. Kisspeptin is a hypothalamic peptide under the influence of KISS1 gene. Based on the fact that it affects gonadotropin-releasing hormone (GnRH) secretion, it is thought to play a role in the development of PCOS. Furthermore, kisspeptin may be determined as a possible biomarker for this syndrome. Due to the complex relationship between kisspeptin and the HPG axis, we aimed to reveal the association of serum kisspeptin level with PCOS in women.

Material and Methods: The study was designed as a prospective study in Koru Ankara Hospital. A total of 88 women were included in the study. Forty four of them were diagnosed with PCOS according to the Rotterdam criteria. Serum kisspeptin measurements were performed using an enzyme-linked immunosorbent assay kit.

Results: : In the present study, PCOS patients showed statistically significant lower serum kisspeptin levels compared to controls.

Conclusion: Although kisspeptin is thought to play a role in PCOS by irregularly stimulating GnRH neurons, the definite mechanism remains still unclear. Clarifying the underlying role of kisspeptin in PCOS may provide valuable information for the future gene treatments.

Keywords: Kisspeptin, luteinizing hormone, neuropeptide, polycystic ovarian syndrome, reproduction.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders, affecting 10–25% of women in reproductive age.[1] Although its pathophysiology is not clarified yet, it is thought to develop from abnormal pulsatile secretion of gonadotropin-releasing hormone (GnRH) leading to impaired follicular functions.[2]

Kisspeptins are a group of peptides expressed by KISS1 gene that functions through G-protein receptor named GPR54.[3] KISS1 gene was first described as a suppressor of metastasis in human melanoma, and kisspeptin release was shown to be altered by it.[4] Kisspeptin was reported to be found in infundibular nucleus of hypothalamus,[5] in ovary, testis, vascular system, pancreas, and placenta.[6] KISS1 gene was shown to play a critical role in ovarian cyclical activity.[4] Kisspeptin is known to be role in the onset of puberty, ovulation, reproduction, and fertility.[7] It binds to GPR54 receptor and potentially stimulates the release of GnRH into circulation that leads to the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland.[8] Beside its hypothalamic effect, kisspeptin was revealed to affect peripheral gonadal secretion of estrogens, progesterone, and androgens.[8] However, irregular gonadotropin release in PCOS might be a result of altered stimulation of GnRH by the peptide kisspeptin.

Referring to complicated relationship between kisspeptin and hypothalamic–pituitary–gonadal (HPG) axis, this study was performed to clarify the association between kisspeptin and PCOS.

MATERIAL AND METHODS

The cases of the present prospective study were recruited from Koru Ankara Hospital, Department of Obstetrics and Gynecology, between June 2022 and August 2022. A total of 88 women were admitted to the study. Forty four of them were diagnosed with PCOS, while the remaining 44 healthy women comprised the control group.

A routine assessment including detailed history, body mass index (BMI), day 1–3 FSH, LH, and estradiol (E2) (following a spontaneous or progesterone induced bleeding) serum tests was performed. Antral follicles of the participants of the two groups were counted by a transvaginal ultrasound. Patients whose symptoms manifested from adolescent years were diagnosed as PCOS according to the Rotterdam criteria.[9] A menstrual cycle was considered oligo-anovulation in the case of a menstrual cycle length of >35 days. Polycystic ovarian morphologic features are defined as at least one ovary with 12 or more follicles between 2 and 9 mm in diameter. Ultrasound examinations were performed by a single gynecologist.

The participants of the control group were selected from the patients who referred to our gynecology department with the complaint of menstrual irregularities. Normo-androgenic, normal cycling before recent irregularities, non-medicated, consenting women of reproductive age in whom PCOS was objectively excluded by clinical, biochemical, and ultrasound assessment were recruited to participate in the study as controls.

Patients who had hypothyroidism, congenital adrenal hyperplasia, and whose taking corticosteroid, antiepileptic, or antipsychotic drugs were excluded from the study. Having the history of hormonal contraception within the previous 6 months and having pregnancy were also the reasons for exclusion from the study. Moreover, participants who had chronic hypertension, type 1 diabetes mellitus, or morbid obesity were excluded. Besides, smokers and women who had undergone any surgical intervention were excluded. The study protocol was approved by the Ethical Committee of Ankara City Hospital (E2-22-1871). All the participants provided written informed consent.

About 10 ml of venous blood sample on menstruation day 3 was taken from each subject and dispensed into lithium heparin. After obtaining serum, then they were stored at -80°C until analysis. Serum kisspeptin measurements were performed using an enzyme-linked immunosorbent assay kit (Phoenix Pharmaceuticals Inc., Belmont, CA, USA). The plate had been pre-coated with human kisspeptin antibody. Kisspeptin present in the sample was added and binded to antibodies coated on the wells. And then, biotinylated human kisspeptin antibody was added and binded to kisspeptin in the sample. Substrate solution was then added and color developed in proportion to the amount of human kisspeptin. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

The demographic characteristics of the participants were recorded. SPSS version 25.0- software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was used for data analysis. Descriptive statistics included mean, standard deviation, median, and interquartile range. Levene's test was used to test for homogeneity of the variances. The Shapiro–Wilk test was used to check whether continuous data followed a normal distribution. Student's t-test was used to analyze the difference between the groups. Nonparametric Mann–Whitney U-test was used for non-normally distributed variables. A $p<0.05$ was considered to be statistically significant.

RESULTS

Forty-four women with the diagnosis of PCOS and 44 healthy women included in the study. Women with the BMI ranged from 17 to 33 were included in both of the groups. BMI ranged from 17 to 30 in control group while it ranged from 18 to 33 in PCOS group. Demographic characteristics and hormone levels of the patients were shown in Table 1. There was no significant difference between the groups in terms of age (years) (U=-1.908, p=0.056) (Table 1). The groups had statistically significant differences in LH/FSH (U=-7.708, p=0.001), E2 (Estradiol) (U=-2.237 p=0.025), mean platelet volume (MPV) (U=-2.232, p=0.026), and BMI (t=3.680 p=0.001) (Table 1). The means of serum levels of LH/FSH and E2 were significantly higher in PCOS patients than in controls (Table 1). The mean BMI was significantly higher in the PCOS group than in the control group. In PCOS group, serum MPV levels were significantly lower than the control group.

When the groups were analyzed according to kisspeptin levels, serum kisspeptin levels were found significantly lower in PCOS group than in the control group (U=-1.995, p=0.049) (Table 2). Therefore, kisspeptin might be an independent marker for PCOS.

Table 1: Clinical characteristics of the study participants (n=88)

	Polycystic ovarian syndrom Mean±SD	Control Mean±SD	Critic value (U)	p
Age (years)	29.18±4.848	30.84±5.103	-1.908	0.056
	28 (26;31)	31 (27;35)		
LH/FSH	2.26±1.258	0.76±0.23	-7.708	0.001**
	2 (1;2)	1 (1;1)		
E2	74±55.969	46.06±25.794	-2.237	0.025*
	50 (37;99)	39 (33;55)		
MPV	10.04±0.752	10.4±0.522	-2.232	0.026*
	10 (9;11)	11 (10;11)		
BMI	25±3.596	22.34±3.169	3.680	0.001**
	25 (22;28)	22 (20;25)		

*: P<0.05; **: P<0.01; SD: Standard deviation; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; E2: Estradiol; M: Median; Q1: 1st quarter; Q3: 3rd quarter; MPV: Mean platelet volume; BMI: Body mass index.

Table 2: Clinical characteristics of the study participants (n=88)

	Polycystic ovarian syndrome Mean±SD M (Q1;Q3)	Control Mean±SD M (Q1;Q3)	Critical value	p
Kisspeptin mean_OD (optical density) value (pg/mL) (mean±SD)	0.54±0.103	0.59±0.111	-2.024	0.0432*
	1 (0;1)	1 (0;1)		
Kisspeptin mean_concentration	18.69±93.953	222.83±101.659	-1.995	0.0491*
	163 (108;239)	239 (141;295)		

*: P<0.05; **: P<0.011; t-test (t)2: Mann–Whitney U-test (U), SD: Standard deviation; M: Median; Q1: 1st quarter; Q3: 3rd quarter.

DISCUSSION

PCOS is a frequently seen endocrine disease affecting almost 10% of women in reproductive ages[10] and it is complicated by several reproductive and metabolic problems. Although pathogenesis is based on both genetic and environmental factors, it is not still clear and a single cause cannot be accused of. Defect in oocyte apoptosis is known to be the underlying cause of the disease.[11]

Kisspeptin is revealed to be a potent regulator of GnRH and gonadotropin release.[8] Kisspeptin binds to GPR54 receptor and participates in the control of HPG axis.[12,13] By binding to its receptor, kisspeptin stimulates the release of GnRH into portal circulation, which in turn stimulates the secretion of LH and FSH from the anterior pituitary.[14] Reversed gonadotropin ratio in PCOS might be a conclusion of an altered kisspeptin inputs to GnRH neurons.[4]

Several studies have been carried out to determine the role of kisspeptin in PCOS. Hu et al.[15] investigated the role of KISS1 and KISS1 receptor (KISS1R) in human granulosa lutein cells to reveal the pathogenesis of PCOS. Due to the fact that the pre-ovulatory follicle number in PCOS was higher than the control group, the expression of KISS1 was detected increased in PCOS. They concluded that the expression levels of KISS1 and KISS1R were significantly higher in the PCOS group than those in the non-PCOS group. Gorkem et al.[10] revealed that serum kisspeptin levels were increased in women with PCOS. Furthermore, they detected that kisspeptin levels were negatively correlated with serum FSH levels and positively correlated with serum total testosterone levels. Araujo et al.[2] compared kisspeptin levels in women with PCOS and with controls. According to their review of the literature, they concluded that the serum kisspeptin levels were higher in women with PCOS than in controls.

Another review of 12 studies includes 660 PCOS patients and 600 controls. It was reported that the kisspeptin levels were lower in the control group than PCOS group.[16] Based on their meta-analysis, Liu et al.[17] declared that serum kisspeptin levels were higher in PCOS patients than controls. They also pointed that kisspeptin might be a biomarker of PCOS. Another study was performed in Baghdad, Iraq and kisspeptin level was found significantly increased in women with PCOS. The authors found a cut-off level of kisspeptin as 189

pg/mL and even claimed that the neuropeptide kisspeptin could be used to diagnose PCOS when its level was higher than 189 pg/mL.[18] Perez Lopez et al.[19] reviewed 18 studies and declared that circulating kisspeptin levels were significantly higher in 1282 PCOS cases than in 977 women without the syndrome.

İbrahim et al.[20] reported in their study that serum kisspeptin levels were higher in PCOS patients than those in the normal group and they also declared that kisspeptin levels increased with age. Umayal et al.[4] indicated that Sri Lankan women with PCOS had significantly higher kisspeptin levels than controls in their study. Furthermore, they suggested that increased kisspeptin levels could be used as an early marker of PCOS to recognize it from adolescence. McCarthy et al.[21] reported that administration of kappa receptor agonists decreases the level of LH by inhibiting Kiss1 neurons in hypothalamic arcuate nucleus in mice. Therefore, they claimed that suppressing the kisspeptin activity by activation of kappa receptors might treat PCOS.

In contrary to the literature, Emekçi Özay et al.[22] reported in their study that the serum levels of kisspeptin did not differ significantly between PCOS and healthy women.

Diversity of PCOS phenotypes may also result in different metabolic findings including normal kisspeptin levels. Panidis et al.[23] and Albalawi et al.[24] also reported that kisspeptin level did not increase in all subtypes of PCOS in their study. They noticed a positive correlation between kisspeptin level and LH, however, they did not find any difference in terms of kisspeptin level in PCOS women compared with control group.[22,24]

In contrary to the majority of the studies in the literature, we found significantly lower kisspeptin levels in PCOS group than the control group. Although it can be argued that the relatively small sample sizes may explain our current contradictory findings, it is noteworthy that our contradictory results may be due to the ethnic origin of woman affecting on the expression of kisspeptin. In addition to this, demographic characteristics of differing study populations may also lead to different results. Furthermore, any genomic interaction might alter serum kisspeptin levels by affecting the expression of KISS1 gene. Moreover, for some PCOS patients, hypothalamic overactivity might be less severe than ovarian dysfunction, and it is known that pituitary and ovarian dysfunction may also lead to the occurrence of PCOS. Since kisspeptin has rarely been reported to play a role in the ovary, it may also explain why kisspeptin levels were not found higher in some of the women with PCOS like in our study. Furthermore, the phenotype of which with normal or lower kisspeptin levels might be in high proportion among PCOS group in our study and this might explain the lower kisspeptin concentration of the whole PCOS group than the control one.

CONCLUSION

To the best of our knowledge, this study is the first to detect a statistically significant decrease in kisspeptin levels of women with PCOS compared to controls. Although many authors propose using increased kisspeptin levels as an early marker of PCOS, the mechanism of kisspeptin for regulating gonadotropin is still unclear and polymorphism of KISS1 gene is not well explained. Therefore, large-scaled studies are needed to clarify whether kisspeptin is reliable to diagnose PCOS or not.

Statement

Ethics Committee Approval: The Ankara City Hospital Clinical Research Ethics Committee granted approval for this study (date: 27.05.2022, number: E2-22-1871).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – İU; Design – İU, AJ; Supervision – İU; Resource – İU, AJ; Materials – İU; Data Collection and/or Processing – İU, AJ; Analysis and/or Interpretation – İU; Literature Search – İU; Writing – İU; Critical Reviews – İU, AJ.

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