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Circulating Kisspeptin and Klotho Levels in Women with Hyperprolactinemia

ABSTRACT

Objective: Kisspeptin is a neuropeptide that acts mainly on gonadotropin-releasing hormone in the hypothalamus with neurokinin B and dynorphin. Also, its peripheral effects have gained importance in recent years. Klotho is an anti-aging transmembrane glycoprotein with growth hormone and insulin-like growth factor-1-related effects. Both kisspeptin and klotho seem to interact with prolactin in the hypothalamic-pituitary region. In this study, we aimed to investigate the relationship between hyperprolactinemia and circulating kisspeptin and klotho levels.

Methods: In this study, 33 women with hyperprolactinemia and 28 control subjects were included. Blood samples were analyzed for follicle-stimulating hormone, luteinizing hormone, estradiol, prolactin, α-klotho, kisspeptin, and neurokinin B levels.

Results: There was no difference between the groups in terms of kisspeptin and α-klotho levels ($P = .68$ and $P = .11$, respectively). Follicle-stimulating hormone and estradiol were significantly lower in the hyperprolactinemia group ($P = .02$ and $P = .03$, respectively). Kisspeptin levels were positively correlated with follicle-stimulating hormone levels ($r = 0.43$, $P = .01$) and α-klotho levels ($r = 0.32$, $P = .01$). Neurokinin B was negatively correlated with both kisspeptin and α-klotho values ($r = -0.42$, $P = .01$ and $r = -0.29$, $P = .02$, respectively). Body mass index was positively correlated with kisspeptin and negatively correlated with α-klotho molecule ($r = 0.273$, $P = .03$ and $r = -0.25$, $P = .04$, respectively).

Conclusion: Hyperprolactinemia does not affect circulating kisspeptin and α-klotho levels but positively correlates with kisspeptin and α-klotho, which are known to play a role in complex interactions between the hypothalamus and the pituitary that may be the subject of new studies in the future.

Keywords: Hyperprolactinemia, kisspeptin, klotho, neurokinin B, pituitary

Introduction

Kisspeptin is a neuropeptide encoded by the tumor suppressor gene Kiss1, and its target receptor is G protein-coupling receptor-54 (GPR54). G protein-coupling receptor-54 functions through the protein kinase C (PKC) intracellular signaling system.[1] Kisspeptin plays an important role in the pulsatile GnRH release and in the regular functioning of the pituitary–gonadal axis by forming a neural network (KNDy) with neurokinin B (NKB) and dynorphin in the hypothalamus.[2] Recent studies have shown that neurokinin B can affect GnRH neuronal activity directly and indirectly. Kisspeptin is the most potent secretagogue for GnRH, while neurokinin B stimulates kisspeptin to initiate GnRH pulse.[3] The proper functioning of this axis is important for timely and effective entry to puberty, as well as for regular menstrual cycle and fertility in women.[4] Prolactin has a suppressive effect on GnRH. Due to hyperprolactinemia, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are suppressed.[5] In women, hyperprolactinemia is associated with hypogonadotropic hypogonadism, amenorrhoea, and infertility.[6]

Although prolactin receptors are present in most kisspeptin neurons, they have only been found in a few GnRH neurons.[7] This suggests that the effects of prolactin on GnRH neurons may be mediated by kisspeptin. Animal studies on the interaction of prolactin and kisspeptin have shown that the expression and activity of kisspeptin receptors in the hypothalamus increase with prolactin infusion.[5,8] In an animal study, menstruation was achieved with kisspeptin infusion in amenorrhoeic mice with hyperprolactinemia.[9] In human and animal studies, an increase in LH and prolactin levels was observed with kisspeptin infusion in the preovulatory period.[10,11]

In recent years, the significance of Klotho, a transmembrane glycoprotein, has increased considerably. It is known that premature aging, atherosclerosis, vascular calcification, genital

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atrophy, skin atrophy, and osteoporosis have developed in the synthesis defect of Klotho enzyme.[12] There are 3 forms of the Klotho protein; alpha, beta, and gamma. The alpha form is the primary responsible form for the effects of this enzyme. Membrane form, 1 of the 2 forms of alpha-Klotho, interacts with fibroblast growth factor-23 to produce a phosphaturic effect in the kidney.[13] The other form, the soluble form, passes into the circulation and functions in the brain (especially the choroid plexus), pituitary, parathyroid glands, and to a lesser extent, in the thyroid, pancreas, ovary, and testis.[14] Studies have shown that an increase in growth hormone (GH) is accompanied by an increase in Klotho levels in patients with acromegaly.[15] In addition, low GH levels are accompanied by low Klotho levels in patients with growth retardation.[16] With these results, it has been suggested that one of the sources of alpha-Klotho may be the pituitary gland and that its synthesis may be GH-dependent or independent.[17] In this context, Klotho levels may be affected by high prolactin levels.

Although there are animal studies on kisspeptin levels in hyperprolactinemia,[5,9,18] to the best of our knowledge, studies examining the interactions of these molecules in humans are limited in the literature. For these reasons, in this study, we aimed to investigate how kisspeptin and klotho levels in the peripheral circulation are affected in women with high prolactin levels.

Material and Methods

All participants were women and were being followed up at Gazi University Faculty of Medicine, Department of Endocrinology and Metabolism, Endocrine Polyclinic. The cut-off value for hyperprolactinemia in the patient group was accepted as 50 ng/mL. Although hyperprolactinemia may vary according to the kits used in laboratories, it is defined as prolactin levels above 25 ng/mL in women. Even though there is no specific cut-off value accepted as pathological level, it is widely accepted in the literature that other common hyperprolactinemic conditions usually cause PRL elevation of <50 ng/mL. In the literature, prolactin levels between 25 and 50 ng/mL are defined as mild prolactin excess which is associated with clinical findings such as short luteal phase, decreased libido, and infertility.[19] Inclusion criteria for the patients were having prolactin levels above 50 ng/mL and not having other common hyperprolactinemic conditions such as non-fasting sample, excessive exercise, recent chest wall surgery or trauma, recent seizure history, and exclusion criteria mentioned below. Inclusion criteria for the control group were having a regular menstrual cycle and normal prolactin level (≤24 ng/mL) and not having any additional diseases that could cause prolactin elevation (e.g., pituitary disease, hypothyroidism, PCOS, etc.). The exclusion criteria for each group were being in a postmenopausal or perimenopausal state, presence of a disease that will cause excess or hypofunction of pituitary hormones other than hyperprolactinemia, presence of a disease other than hyperprolactinemia that may cause primary or secondary gonadal insufficiency (e.g., primary ovarian failure, having undergone pituitary surgery, pituitary radiotherapy or gamma-knife treatment that may affect the FSH–LH–testosterone and estradiol (E2) axis, using an oral contraceptive treatment that may affect the FSH–LH–E2 axis, or using a drug that may cause prolactin elevation (antipsychotic, antidepressant, using alpha methyl dopa, verapamil etc.), using cabergoline or bromocriptine in the last 1 year period, presence of malignancy, having additional chronic disease (chronic kidney disease, chronic liver disease, etc.), presence of a mass that may affect the hypothalamus function, morbid obesity, and being younger than 18 years or older than 50 years old. Before starting the study, the approvals were obtained from the Gazi University Faculty of Medicine Ethics Committee, and written informed consent was signed by all patients.

MAIN POINTS

- Kisspeptine is a molecule that has increased in importance in recent years, and has a significant effect on numerous hormones in the pituitary, hypothalamus and periphery, especially gonadotropins and prolactin.
- Klotho is an anti-aging protein whose source is not exactly known in the body, but there are studies that the main source may be pituitary tissue.
- Kisspeptin and klotho levels in hyperprolactinemia patients were investigated in the article.

Anthropometric and Clinical Metrics

All participants' medical history and physical examination were recorded. Body mass index (BMI) (kg/m^2) was calculated by measuring height and weight of all participants.

Biochemical and Hormonal Measurements

Blood samples were taken from the participants from the antecubital vein between 8:00 and 10:00 AM, after 8 hours of fasting and between the second and fifth days after the onset of menstruation. Totally 21 patients with hyperprolactinemia had menstrual cycle disruption, mostly oligomenorrhea. Blood sample was also obtained between the second and fifth days after the onset of menstruation from the patients with menstrual cycle disruption. Macroprolactinemia was excluded in all patients with hyperprolactinemia. The blood samples were centrifuged and stored in a −80°C freezer after the serum was separated. Follicle-stimulating hormone, luteinizing hormone, E2, and prolactin analysis were performed by chemiluminescent immunoassay method; the levels of kisspeptin, α-klotho, and neurokinin B were studied by enzyme-linked immunosorbent assay (ELISA) method. Human Kiss1 ELISA kits were used for kisspeptin measurement and NKB ELISA kits (USA Elabscience Laboratory, 14780 Memorial Drive, Suite 216, Houston, Texas 77079) were used for NKB measurement. The Kisspeptin kit sensitivity was 75 pg/mL and the measuring range was 125-8000 pg/mL. The Human NKB ELISA kit sensitivity was 9.38 pg/mL and the measuring range was 15.63-1000 pg/mL. Human SAKL (soluble alpha-klotho) ELISA kits (Mybiosource Laboratories, San Diego, USA) were used for alpha-klotho measurements. The alpha-klotho kit sensitivity was below 56.25 pg/mL and the measuring range was 93.75-6000 pg/mL. All these kits were specific to human levels and did not have analogs that would cause cross-reaction or interference.

Statistical Analysis

The results of normal distribution tests in the groups were evaluated by Kolmogorov–Smirnov method. Non-parametric parameters' median and 25th-75th interquartile ranges were given. Mann–Whitney *U* test was used to compare non-parametric independent variables. Spearman correlation test was performed to investigate the correlation between Kisspeptin, Klotho, and other parameters in hyperprolactinemia, control, and all participants groups.

Table 1. The Demographic and Biochemical Results of the Hyperprolactinemia and Control Groups

	Hyperprolactinemia Group (n = 33)	Control Group (n = 28)	*P*
Age (years)	30 (25-42.5)	34 (28.25-39.75)	.39
BMI (kg/m^2)	24.9 (22.2-28.16)	25.07 (21.28-28.59)	.87
PRL (ng/mL)	**91 (61.5-130)**	**10.21 (7.22-16.56)**	**.01**
FSH (mIU/mL)	**6.42 (5.38-8.57)**	**7.98 (7.05-9.45)**	**.02**
LH (mIU/mL)	5.27 (3.62-7.21)	4.93 (4.10-7.27)	.87
E2 (pg/mL)	**46.75 (29.24-55.45)**	**56.50 (40-87.25)**	**.03**
Kisspeptin (pg/mL)	1309 (1061-2069)	1304 (1063-1593)	.68
NKB (pg/mL)	56.42 (50.24-77.61)	73.74 (50.8-91.96)	.26
Klotho (pg/mL)	906.3 (775.6-1193)	831.5 (725.9-1034)	.11

Mann–Whitney *U* test was used, median values and %25th and 75th interquartile ranges were given. $P < .05$ was accepted to be statistically significant. Number in bold indicate statistically significant results.
BMI, body mass index; PRL, prolactin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; NKB, neurokinin B.

Results

The study included 33 hyperprolactinemia patients and 28 healthy control subjects. The age, BMI, and biochemical outcomes are summarized in Table 1. In terms of age and BMI, there was no significant difference between the 2 groups ($P = .39$ and $P = .87$, respectively). As expected, prolactin level was found to be significantly higher in the patient group ($P = .01$). Follicle-stimulating hormone and E2 levels were found to be significantly low in the patient group ($P = .02$ and $P = .03$, respectively). No significant differences were detected between the Kisspeptin, NKB, klotho, and LH levels ($P > .05$).

Pituitary magnetic resonance imaging results for hyperprolactinemia revealed microadenoma in 23 patients, macroadenoma in 1 patient, and heterogeneity in the pituitary (no obvious adenoma formation) in 9 patients. The results of the correlation analyses between kisspeptin and Klotho and other parameters in hyperprolactinemia, control groups, and all participants are shown in Tables 2-4. There was a statistically significant positive correlation between kisspeptin and FSH in all correlation analyses. A statistically significant negative correlation was observed between kisspeptin and NKB. Klotho also had negative correlation with NKB. In all participants' correlation analysis, kisspeptin showed a positive correlation with BMI, while Klotho showed a negative correlation. Kisspeptin and Klotho had a statistically significant positive correlation in all groups.

Table 2. Evaluation of Correlation Analyses Between Kisspeptin and Other Parameters in Hyperprolactinemia and Control Groups

	Hyperprolactinemia Group (Kisspeptin)		Control Group (Kisspeptin)	
	r	*P*	*r*	*P*
Age	0.04	.81	0.292	.13
BMI	**0.339**	**.02**	0.205	.29
PRL	0.203	.21	−0.174	.37
FSH	**0.376**	**.03**	**0.614**	**.01**
LH	0.08	.62	0.03	.85
E2	0.123	.49	0.334	.08
NKB	**−0.434**	**.01**	**−0.375**	**.04**
Klotho	**0.408**	**.01**	**0.201**	**.03**

$P < .05$ was accepted to be statistically significant. Number in bold indicate statistically significant results.
BMI, body mass index; PRL, prolactin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; NKB, neurokinin B; *r*, Spearman rho value.

Table 3. Evaluation of Correlation Analyses Between Klotho and Other Parameters in Hyperprolactinemia and Control Groups

	Hyperprolactinemia Group (Klotho)		Control Group (Klotho)	
	r	*P*	*r*	*P*
Age	0.09	.60	−0.185	.28
BMI	−0.137	.07	**−0.358**	**.03**
PRL	0.166	.36	−0.017	.92
FSH	0.264	.14	−0.137	.43
LH	−0.07	.68	0.017	.92
E2	0.196	.28	0.213	.21
NKB	**-0.506**	**.01**	**−0.137**	**.04**
Kisspeptin	**0.415**	**.01**	**0.144**	**.03–**

$P < .05$ was accepted to be statistically significant. Number in bold indicate statistically significant results.
BMI, body mass index; PRL, prolactin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; NKB, neurokinin B; *r*, Spearman rho value.

Discussion

In our study, we have demonstrated that an increase in the prolactin level has no effect on peripheral kisspeptin and klotho levels in women. In recent years, research and resulting data on the relationship between prolactin and kisspeptin have been increasing rapidly. In animal studies, it has been shown that there are prolactin receptors in neurons expressing Kiss1[7,20] and that interactions occur between prolactin and kisspeptin through these receptors.[5,8,18] An animal study has found a reduction in the immunoactivity of kisspeptin in the hypothalamic kiss1 mRNA along with the high prolactin levels in the arctic and anteroventral paraventricular neurons.[9] In another animal study, prolactin infusion did not change the levels

Table 4. Evaluation of Correlation Analyses Between Kisspeptin, Klotho, and the Other Parameters in All Participants

	Kisspeptin		Klotho	
	r	*P*	*r*	*P*
Age (years)	0.114	.38	−0.43	.74
BMI (kg/m^2)	**0.273**	**.03**	**−0.257**	**.04**
PRL (ng/mL)	0.07	.56	0.113	.10
FSH (IU/L)	**0.430**	**.01**	−0.025	.33
LH (IU/L)	0.05	.69	−0.042	.16
E2 (ng/L)	0.157	.22	0.206	.41
NKB (pg/mL)	**−0.425**	**.01**	**−0.292**	**.02**
Klotho (pg/mL)	**0.325**	**.01**	-	-
Kisspeptin (pg/mL)	-	-	**0.325**	**.01**

$P < .05$ was accepted to be statistically significant. Number in bold indicate statistically significant results.
BMI, body mass index; PRL, prolactin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; NKB, neurokinin B; *r*, Spearman rho value.

of the central nervous system kisspeptin.[20] However, a human study conducted through kisspeptin infusion in men and women found that the effect of kisspeptin on the follicular phase was present but limited.[21] In the study by Kotani et al[22], no significant difference was found between peripheral kisspeptin levels of women with high prolactin levels in the lactation period and normal premenopausal women. Molecular and animal studies have demonstrated that prolactin is affected by multiple molecules such as dopamine and thyrotropin and that particularly E2 levels are decisive in interactions between kisspeptin and prolactin.[5,20,23] In cases where E2 is relatively low, such as follicular phase, the interactions of kisspeptin prolactin are less potent.[21,24] We consider that the lower E2 levels in the hyperprolactinemia group may reduce the effect of prolactin on kisspeptin molecule. In addition, considering that most of the studies on prolactin and kisspeptin interactions were done on the central nervous system, it is plausible that the pathophysiology observed in the hypothalamus does not fully reflect on the kisspeptin levels in the peripheral circulation.

We demonstrated that, in the hyperprolactinemia group, FSH and E2 levels are significantly lower than in the control group, and there is no difference between LH levels. This might be related to the suppressive effect of high prolactin levels on GnRH. Thus, FSH and LH levels decrease and the loss of overstimulation leads to gonadal dysfunction.[24,25] The results of FSH and E2 are consistent with the literature and the fact that there is no difference in LH levels between groups is attributed to the analysis of LH levels in the early follicular phase of the menstrual cycle.

In the correlation analysis with kisspeptin, a moderate correlation with FSH was found which is in concordance with normal physiology. Also, a recent study which examined the direct effects of kisspeptin on basic ovarian cell functions and their response to FSH found that the addition of FSH to the kisspeptin increased ovarian cell viability, proliferation, decreased apoptosis, and promoted progesterone, testosterone, and E2 which demonstrated the functional interrelationships between kisspeptin and FSH in the direct regulation of ovarian functions.[26] This relationship may reveal a new dimension in the evaluation of the correlation between FSH and kisspeptin that we detected in peripheral blood in our study. Kisspeptin stimulates the secretion of both LH and FSH in human, although the effect on the former is more marked.[27] In studies, it has been shown that plasma administration of kisspeptin in the periovulatory and luteal phase in women stimulates the release of LH, but in the follicular phase of menstruation, responses to exogenous kisspeptin infusion showed variability, and results including animal studies showed that the response of LH to kisspeptin in the follicular phase is less.[21,28] In our opinion, this concept may help to explain the lack of correlation between kisspeptin and LH in our study.

Neurokinin B was negatively correlated with both kisspeptin and klotho. Compelling evidence suggests that NKB plays a critical role in the control of kisspeptin release, at least at the level of the arcuate nucleus.[29] A clinical study of NKB administration showed that none of the doses of NKB tested were associated with significant alterations in reproductive hormone secretion in healthy female volunteers.[30] Neurokinin B and kisspeptin work together in the central nervous system, but their interactions in the periphery seem to be limited. We can attribute this to the fact that there is an opposite relationship between NKB and kisspeptin in the periphery in our study.

Most of the participants in our study were overweight and there were no patients diagnosed with diabetes and we demonstrated that there was a positive correlation between BMI and kisspeptin levels. Along with hypothalamus and pituitary, Kiss1r and the kisspeptin receptors have been reported in peripheral tissues including adipocytes, heart, spinal cord, gonads, and pancreas, and kisspeptin is known to be expressive in adipose tissue.[31] In recent years, studies of the peripheral effects of kisspeptin have been increasing considerably. There are studies that demonstrated positive[31,32] and also negative correlations[33,34] between kisspeptin and BMI. It should be taken into consideration that in most of these studies, kisspeptin expression in adipose tissue was primarily investigated, and often the status of diseases that affect insulin and fat metabolisms, such as diabetes and obesity, is not detailed.

In our study, we found that high prolactin levels did not affect the circulating klotho level. Studies investigating pre- and post-surgical Klotho levels in GH-secreting pituitary tumors have shown that there are high levels of Klotho with high GH levels before surgery and that Klotho levels decrease with a decrease in GH levels after surgery.[15,17] Interestingly, also in non-functional pituitary adenomas, a decrease in Klotho levels after surgery was observed. Based on these results, they suggested that the pituitary gland may be one of the sources of klotho in the body.[17] In our study, most of the prolactin-secreting adenoma sizes were below 1 cm, and the mean values of prolactin in the patient group were moderately high.

Klotho and BMI were negatively correlated in our study and we found a significant positive correlation between kisspeptin and klotho. Recent data showed that centrally circulating α-klotho modulates arcuate cell populations in mice and demonstrated strong inverse correlations with body weight and an ability to increase energy expenditure.[35] Although there is no clear pathophysiological link between kisspeptin and klotho, further investigation of these novel molecular mechanisms could be the key to deciphering the complex physiology underlying the regulation of hormonal metabolism.

There are limitations to this study. The fact that adenoma formation is not clear in all patients despite the presence of prolactin elevation, the mean prolactin level is moderately high, and the molecules examined can only be studied in peripheral blood can be counted among these limitations. The study did not include GH and IGF-1 levels, and their correlation analysis with Klotho, which, if added, could give a different perspective to this subject. Increasing the number of patients and prolonging the follow-up period and re-performing the examinations in patients who have been cured after hyperprolactinemia treatment may increase the power of the study. Due to the fact that the response to treatment had a share in the etiology of hyperprolactinemia, there were patients whose etiology of hyperprolactinemia was not clearly demonstrated as a result of the lack of long-term follow-up. However, to the best of our knowledge, this is one of the few studies in the literature that examines the relationship between prolactin, kisspeptin, NKB, and klotho, and a positive correlation between Kisspeptine and Klotho is revealed for the first time.

As a result, in women with hyperprolactinemia, circulating kisspeptin, NKB, and klotho levels are not affected. This situation may be the result of the fact that the pathophysiology in the central nervous system is not fully reflected in the peripheral circulation. Nevertheless, the newly identified interactions in this complex mechanism, such as

the kisspeptin and klotho correlation, suggest that there is more to be investigated on this matter.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Gazi University Faculty of Medicine.

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

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