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An assesment of relationship between serum preptin levels and anti-mullerian hormone in infertile womens with polycystic ovary syndrome

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Abstract

Aim: Despite the clear relationship between AMH, insulin and androgen levels, the relationship between preptin and AMH levels is not known. The close metabolic similarity between preptin and insulin suggests that there may be a relationship between this peptide and AMH. This study was planned to reveal the relationship between serum preptin, AMH, insulin and other metabolic parameters in infertile women with polycystic ovary syndrome (PCOS).

Material and Methods: Thirty infertile women with PCOS and 30 age- and BMI- matched control patients who did not have clinical and laboratory findings of PCOS were included in the study. In addition to serum preptin and AMH levels, other hormonal parameters such as insulin, androgens and HOMA-IR were measured on the third day of the cycle. Human preptin ELISA kit was used for preptin measurement. AMH Gen II ELISA kit was used for AMH measurement. The correlation between AMH, preptin, HOMA-IR and other endocrine and demographic parameters were evaluated.

Results: Serum total testosterone, insulin, LH levels and HOMA-IR of patients in the PCOS group were significantly higher than in the non-PCOS control group. FSH levels were similar in both groups. Median AMH levels in the PCOS groups were 8.44 (3.40–18.34) ng/mL. Median AMH levels in the non-PCOS control group were 3.50 (1.80–8.13) ng/mL. AMH values in the PCOS group were found to be significantly higher than in the control group ($p<0.001$). Serum preptin levels in the PCOS group were approximately six times higher than in the control group (244.13 (27-367) pg/mL vs. 42.22 (13-56) pg/mL, $p <0.002$). A positive and significant correlation was found between serum preptin levels and AMH ($r=0.76$, $p <.05$) and HOMA-IR ($r=0.69$, $p <.02$) values. A positive and significant correlation was found between AMH levels and both serum LH ($r=0.70$, $p <.05$) and testosterone levels ($r=0.88$, $p <.01$).

Discussion: This clinical study showed for the first time a positive relationship between serum preptin levels and AMH values in PCOS patients.When our study and the literature were reviewed together, it was observed that serum preptin levels increased in PCOS patients with a clinical picture similar to metabolic syndrome. Preptin's relationship with other endocrine and demographic parameters is similar to insulin. In our study, the only point different from other studies is the positive correlation between preptin and serum AMH values. Despite the small number of our cases, this study has clinical significance as it is the first study to examine the relationship between preptin and AMH and insulin levels. We will be able to make clearer comments on this issue thanks to studies investigating serum and intra-follicular AMH and preptin values in PCOS cases simultaneously.

Keywords
PCOS, HOMA-IR, Preptin, AMH, Insulin

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Introduction

Anti-Mullerian hormone (AMH) is a glycoprotein hormone that is synthesized locally by granulosa cells but provides clear information about the ovarian reserve by entering the systemic circulation [1]. Serum AMH levels are significantly higher in anovulatory PCOS patients than in normo-ovulatory women [2]. AMH is one of the 35 different members of the transforming growth factor-β superfamily [3] and shows its basic functions in the ovaries and other reproductive organs [4]. AMH prevents premature recruitment and maturation of follicles by blocking the stimulatory effect of FSH on the aromatase enzyme [5]. The gene encoding AMH is on the short arm of chromosome 19 [6], and the ability of granulosa cells to synthesize AMH decreases with age and decreases to undetectable levels in circulation after menopause [7]. Although there are fluctuations in AMH levels in different phases of the cycle, these changes are not significant enough to affect the amount of AMH to be measured [8]. In addition to the increase in AMH mRNA levels, the increase in both follicle number and granulosa cell mass are the main causes of AMH elevation in PCOS cases [9].

Preptin is a peptide secreted from the pancreas together with insulin and stimulates insulin secretion. Similar to insulin, glucose stimulates preptin release from pancreas [10]. A correlation between insulin resistance and serum preptin levels has been reported in PCOS patients [11,12]. Celik et al. showed that serum preptin levels in PCOS cases were significantly higher than in healthy controls [12]. Despite increasing serum preptin levels in PCOS cases, follicular fluid preptin levels have been reported to be low and it has been interpreted that this may contribute to the anovulation seen in PCOS [13]. The correlation between serum AMH levels and hyperandrogenism and LH is a known fact [14,15]. Similarly, a positive correlation was reported between AMH values and insulin levels, and insulin resistance [16]. Until now, there has been no study investigating the relationship between serum AMH and preptin levels in PCOS cases. Despite the clear relationship between AMH, insulin and androgen levels, the relationship between perptin levels and AMH is not known. The close metabolic similarity of preptin to insulin suggests that there may be a relationship between this peptide and AMH. This study was planned for the first time to reveal the relationship between serum preptin, AMH, insulin and other metabolic parameters in infertile PCOS patients.

Material and Methods

Patient selection

This case-controlled study was conducted on 60 patients who applied to the Kayseri City Hospital with the complaint of infertility between May and December 2021. While 30 participants consisted of infertile patients diagnosed with PCOS, the remaining 30 consisted of infertile patients without clinical and laboratory findings of PCOS. Patients in both groups were matched for age and BMI. PCOS was defined according to the revised Rotterdam criteria, which require two of the following three manifestations: (i) oligo-anovulation or anovulation; (ii) high concentrations of androgen in the bloodstream and/or clinical signs of androgen surplus (hyperandrogenism); and (iii) polycystic ovaries shown by ultrasonography. All women in the PCOS group were subjected to progesterone-induced withdrawal bleeding to determine their follicular phases.

Serum samples were taken on the third day of the cycle from the patients in PCOS and control groups for measuring AMH and preptin levels. In addition to demographic characteristics of women in PCOS and control group, age, body mass index (BMI) (kg/m2), total testosterone, fasting glucose, fasting insulin, serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were also measured. Fasting serum insulin levels were also measured in autoanalyzer using electrochemiluminescence immunoassay. Insulin resistance was measured using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) according to the following formula: fasting insulin (mIU/L) × fasting plasma glucose (mg/dL)/405. The study was approved by the local Research Committee of Kayseri City Hospital and all patients signed informed consent before the inclusion.

Preptin Assay

Serum preptin levels were measured using the Human preptin ELISA kit. The measurement was made in accordance with the working procedures defined in the kit catalog. The intra- (within-day) and inter-assay (between days) coefficients of variation for follicular fluid and serum preptin were <8% and <12%, respectively. The minimum detection limit of preptin was 25 pg/mL. The assay range was 5 - 1500 pg/mL.

AMH Assay

After venous blood collection, serum for assay of AMH was separated and frozen. All samples were analyzed using an ultra-sensitive AMH Gen II ELISA kit (Beckman-Coulter, Inc., Webster, NY, USA). The lower limit of AMH detection was 0.16 µg/l. Inter-assay variation was 10% at 0.27 µg/l. All values were expressed in ng/mL.

Statistical Analysis

The Statistical Package for Social Sciences, version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. To assess the normality of data, the Kolmogorov–Smirnov tests were used. While normally distributed data are presented as mean ± standard deviation, non-normally distributed data are presented as median (range). The Mann–Whitney test was used for non-normally-distributed data. Spearman's correlation analysis was used for detecting correlation between AMH, preptin, insulin, HOMA-IR and other measured variables.

Results

The demographic and laboratory findings of each group of participants are shown in Table 1. The patients in the PCOS group and non-PCOS control group were found to be similar in terms of age and body mass index (kg/m2) values. The median AMH level in the PCOS group was 8.44 (3.40–18.34) ng/mL. Median AMH level in the non-PCOS control group was 3.50 (1.80–8.13) ng/mL. AMH values in the PCOS group were found to be significantly higher than in the control group ($p<0.001$). Serum preptin levels in the PCOS group were approximately six times higher than in the control group (244.13 (27-367) pg/mL vs. 42.22 (13-56) pg/mL, $p <0.002$).

A positive and significant correlation was found between serum preptin levels and AMH ($r=0.76$, $p <.05$) and HOMA-IR ($r=0.69$, $p<.02$) values. Likewise, a positive and significant correlation was found between AMH levels and both serum LH ($r = 0.70$,

p <.05) and testosterone levels (r = 0.88, p <.01). However, we could not find any correlation between serum preptin levels and serum testosterone and LH levels. We found a positive and significant correlation between AMH levels and serum insulin levels. Similarly, we found a significant correlation between serum preptin and insulin levels. We found no correlation between serum preptin levels and BMI and patient age. Although we could not find any correlation between AMH levels and BMI values we found a negative and significant correlation between patient age and AMH values.

Table 1. Demographic and hormonal characteristics of subjects with PCOS and non-PCOS

	PCOS (n=30)	Non-PCOS (n=30)	P
Age (y)	28 (21-34)	27 (20-35)	0.06
BMI (kg/m2)	25.1±2.01	24.7±4.08	0.56
Testosterone (ng/dL)	67 (13-68)	27 (16-45)	0.02
LH (mIU/ml)	13 (8-22)	5 (4-11)	0.01
FSH (mIU/ml)	5.1 (2.6-9)	4.8 (3.5-8.2)	0.54
Insulin (mU/L)	13.5±1.05	6.09±4.99	0.01
HOMA-IR	4.3 (0.7-7.9)	2.3 (0.3-4.6)	0.01
Glucose (mg/dL)	93.3±0.35	96.7±1.90	0.10
AMH (ng/mL)	8.44 (3.40–18.34)	3.50 (1.80–8.13)	0.001
preptin (pg/ml)	244.13 (27-367)	42.22 (13-56)	0.002

Glucose, insulin and BMI are presented as means ± SD, others are presented as median (range). AMH; Anti-Mullerian hormone, BMI; body mass index, FSH; follicle-stimulating hormone, HOMA-IR; homeostasis model assessment of insulin resistance, LH; luteinizing hormone, PCOS; polycystic ovary syndrome, *p<0.05.

Discussion

Our study is the first clinical study to show the positive relationship between serum preptin levels and AMH values in PCOS patients. Similar to the results of previous studies, the serum preptin levels of our PCOS patients were found to be significantly higher than in the control group without PCOS clinic. The relationship between serum preptin levels and other endocrine parameters was very similar to the correlation characteristics of insulin found in PCOS patients. Similar to insulin, serum preptin levels were positively correlated with serum levels of AMH. Preptin also showed a significant correlation with serum insulin and HOMA-IR values. Unlike insulin, we could not find a correlation between preptin levels and LH, testosterone BMI and age values of PCOS patients.

In a recent study by Celik et al [12,13] serum and follicular fluid preptin levels of PCOS patients were evaluated, and serum preptin levels were found to be significantly higher than follicular fluid. In the control group, serum and follicular fluid preptin levels were found to be similar. In the light of these data, the authors mentioned the existence of resistance that prevents preptin transport between serum and follicular fluid in PCOS cases. The authors also emphasized that preptin resistance may be associated with anovulation, which is the main finding of PCOS [13]. In our study, while only serum preptin levels were measured, follicular fluid preptin levels were not measured. If there is low intra-follicular preptin concentration and resistance to transfer of serum preptin to the follicle in PCOS as reported in the previous study, how does the stimulatory effect of preptin on AMH occur? Since we have found a positive correlation between preptin and AMH, we think that enough preptin passes from the circulation to the follicle to stimulate AMH levels. However, in order to clarify this idea, studies comparing intra-follicular preptin levels and AMH levels are needed.

We have encountered a total of four studies evaluating preptin levels in PCOS patients [12,13,17,18]. In two studies conducted by Celik et al [12,13], serum preptin levels in PCOS cases were reported to be higher than in healthy controls. Similarly, a positive correlation was found between serum preptin levels and HOMA-IR and androgen levels in these two studies. In a study by Senturk et al [18], no difference was found between PCOS and healthy control groups in terms of serum preptin levels. Mierzwicka et al [17], on the other hand, could not detect a correlation between endocrine parameters and preptin levels, despite high levels of preptin in PCOS. Serum preptin levels were also investigated in cases of gestational diabetes, a metabolic disease similar to PCOS. Aslan et al [19] reported that maternal serum and cord blood preptin increased in GDM patients and correlated with HOMA-IR levels. Aydin et al [20] investigated the plasma and colostrum preptin levels in GDM patients and found high preptin levels in both circulation and colostrum.

When our study and the literature were reviewed together, it was observed that serum preptin levels increased in PCOS patients with a clinical picture similar to metabolic syndrome. Preptin's relationship with other endocrine and demographic parameters is similar to insulin. Preptin is a proinsulin-like growth factor II E-peptide, which is evidence to explain its insulin-like effects [13]. In our study, the only point that differs from other studies is the positive correlation between preptin and serum AMH values. We do not know clearly by what mechanism preptin increases AMH levels. Preptin increases androgen synthesis by making insulin resistance and hyperinsulinemia and stimulates AMH synthesis indirectly in increasing androgens. Despite the small number of our cases, this study has clinical significance as it is the first study to examine the relationship between preptin and AMH and insulin levels. We will be able to make clearer comments on this issue thanks to studies investigating serum and intra-follicular AMH and preptin values in PCOS cases simultaneously.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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