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Association between serum copeptin levels and non-obese normoglycemic polycystic ovary syndrome: A case control study

Serum kopeptin düzeyleri ile obez olmayan normoglisemik polikistik over sendromu arasındaki ilişki: Bir olgu kontrol çalışması

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Abstract

Objective: Copeptin is a glycopeptide that increases under stress and is present in polycystic ovary syndrome (PCOS) patients with metabolic system disorders. We examined the relationship between copeptin and reproductive function in patients with normoglycemic PCOS with anovulatory cycles and normal weight.

Materials and Methods: Women with unexplained infertility (n=52) and women with PCOS (n=57) were included in the study. PCOS was determined using the Rotterdam criteria. Biochemical tests including estradiol, follicle-stimulating hormone, luteinizing hormone, anti-Müllerian hormone (AMH), insulin, and copeptin were performed. Serum copeptin concentrations were measured using enzyme immunoassay.

Results: There were no significant differences in demographic data, insulin levels, and insulin resistance between the PCOS and healthy volunteers. Copeptin levels were lower in the PCOS group ($p<0.001$). A significant negative correlation was observed between AMH and copeptin in the control group ($r=-0.402$, $p= 0.013$). In the PCOS group, a negative correlation was observed between antral follicle count and copeptin, as well as between AMH and copeptin ($r=-0.544$, $p<0.01$). Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value of copeptin levels. The estimated areas under the ROC curves for serum concentration were found to be statistically significant ($p<0.001$) with a cut-off value of 2.78 (95% confidence interval 0.701-0.896), sensitivity of 0.87, and specificity of 0.70.

Conclusion: This study showed that copeptin levels are lower in patients with PCOS in the absence of insulin resistance and obesity than in healthy volunteers, and there is a negative correlation between copeptin and reproductive markers.

Keywords: Copeptin, infertility, PCOS, obesity, insulin resistance, antral follicle

Öz

Amaç: Copeptin stres durumunda artan bir glikopeptid olup metabolik sistem bozukluklarında polikistik over sendromu (PKOS) hastalarında artmaktadır. Anovulatuar siklusları olan ve normal kilolu normoglisemik PKOS hastalarında copeptinin reproduktif fonksiyonlarla ilişkisini incelemeyi amaçladık.

Gereç ve Yöntemler: Çalışmaya açıklanamayan infertil kadınlar (n=52) ve PKOS kadınlar (n=57) alındı. PKOS roterdam kriterleri ile belirlendi. Estradiol, folikül uyarıcı hormon, luteinize edici hormon, anti-Müllerian hormon (AMH), insülin ve copeptin içeren biyokimyasal testler analiz edildi. Serum copeptin konsantrasyonlarının analizleri, enzim immünoassay vasıtasıyla ölçüldü.

PRECIS: Polycystic ovary syndrome is a multifactorial disease that is associated with anovulation and infertility and can include many findings of metabolic syndrome such as insulin resistance. Copeptin is a glycopeptide that increases under metabolic stress. In our study, we evaluated Copeptin levels in patients with Polycystic ovary syndrome who do not have metabolic syndrome findings. We found that Copeptin levels did not increase despite anovulation when there were no metabolic syndrome findings. However, we observed a negative correlation between copeptin and other markers indicating reproductive function.

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Bulgular: PKOS grubunda ve sağlıklı gönüllerde demografik veriler, insülin düzeyleri ve insülin rezistansları arasında fark yoktu. Copeptin düzeyleri PKOS grubunda daha düşük saptandı (p<0,001). Kontrol grubunda AMH ile copeptin arasında anlamlı negatif korelasyon gözlendi (r=-0,402, p= 0,013). PKOS grubunda AMH ve copeptinnin yanı sıra (r=-0,544, p<0,01) Antral folikül sayısı ve copeptin arasında negatif korelasyon gözlendi. PKOS prediktivitesinin belirlenmesi için copeptin düzeylerine ROC analizi yapıldı. Serum konsantrasyonu için alıcı işletim karakteristiği eğrileri altındaki tahmini alanların 2,78'lik (95% güven aralığı 0,701-0,896) bir kesme değeri, 0,87 duyarlılık ve 0,70 özgüllük ile istatistiksel olarak anlamlı (p<0,001) olduğu bulundu.

Sonuç: Bu çalışma PKOS hastalarında insülin rezistansı ve obezite olmadığında copeptin düzeylerinin sağlıklı gönüllülere kıyasla daha düşük olduğunu, copeptin ile reproduktif belirteçler arasında ise negatif yönlü korelasyon olduğunu göstermektedir.

Anahtar Kelimeler: Copeptin, infertilite, PKOS, obezite, insülin rezistansı, antral folikül

Introduction

Polycystic ovary syndrome (PCOS) is a clinical condition characterized by irregular menstruation, anovulation with increased androgen levels, and polycystic ovaries[1]. It is the most common multisystem endocrinological disease in women of reproductive age, affecting approximately 10-13% of the population from adolescence to menopause[2]. Insulin resistance may be observed in patients with PCOS, and this resistance is aggravated by obesity[3]. In patients with PCOS, oxidative stress, proinflammatory cytokine, and adipokine changes arrest follicular development, cessation of corpus luteum functions, anovulation, and hyperandrogenism. Impaired steroidogenesis and ovulation due to hyperandrogenism are the main causes of infertility in PCOS patients[4]. Infertility treatment for patients with PCOS should begin with preconception counseling and weight control, followed by ovulation induction and selection of assisted reproductive treatments[5].

Copeptin is a 39-amino acid glycopeptide that is the C-terminal product of preprovasopressin (pre-proAVP). It is secreted in response to stress and is correlated with plasma arginine vasopressin (AVP) levels[6]. Studies have shown that high serum copeptin levels are associated with obesity, insulin resistance, type 2 diabetes mellitus, hypertension, and hyperlipidemia[7]. The World Health Organization defines obesity as a body mass index (BMI) ≥ 30 kg/m^2. Obesity changes the secretion of various adipokine, such as adiponectin and leptin, and increased leptin levels have been found in women with ovulatory disorders[8]. When obese and non-obese women with PCOS were compared, copeptin levels were found to be higher in obese women[9].

The effects of copeptin on the reproductive system have been shown in patients with poor ovarian reserve, and it has been mentioned that it may have predictive value[10]. However, there are no clinical studies in which the relationship between obesity and copeptin has been eliminated and the reproductive functions of patients with non-obesity PCOS have been evaluated. We aimed to compare copeptin levels between non-obese patients with PCOS and healthy volunteers and to determine the relationship with reproductive markers.

Materials and Methods

Setting

Our research was conducted at Hitit University Faculty of Medicine with participants in the Gynecology and Obstetrics Clinic between 2021 and 2023. The research, which was designed as a case-control study, was carried out prospectively. Research ethics committee and approval were obtained from Hitit University Faculty of Medicine Clinical Research Ethics Committee (decision no: 383, date: 20.01.2021). Women participating in the study were provided with informative information, and written consent for the study was obtained.

Study Population

Gynecological and reproductive anamnesis of the participants were recorded, gynecological examinations were performed, and laboratory techniques were planned. Anamnesis was performed in women included in the study, and physical examination was performed. Patients who had undergone pelvic surgery, medication, or radiotherapy that affected their reproductive functions were excluded from the study. Patients with metabolic syndrome, obese patients (BMI over 30 kg/m^2), and patients with systemic diseases affecting reproduction were not included in the study. Infertile women aged between 20 and 40 years were included in the study, and antral follicle counts were performed by transvaginal sonogram (Toshiba Xario 100) in the early follicular phase of the women's menstrual cycle.

Unexplained infertility is defined as the inability to achieve pregnancy without an explanatory cause despite 1 year of unprotected intercourse. The 109 women included in the study were divided into two groups according to their ovarian reserves. The control group consisted of 52 women, and the women in this group were diagnosed with unexplained infertility. There were 57 women in the study group. The study participants were infertile women diagnosed with PCOS according to the Rotterdam criteria.

Biochemical Evaluations

Blood samples were collected from the antecubital vein. Blood samples collected during the early follicular period of the menstrual cycle were separated into 5 mL tubes (BD Vacutainer, Becton Dickinson, New Jersey, USA). The blood samples were centrifuged at 1000 g for 20 min. Laboratory workers who were unaware of the source of the samples placed the blood samples in an autoanalyzer (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany) for hormonal evaluation and measured follicle-stimulating hormone, estradiol, and luteinizing hormone (LH) levels using the electrochemiluminescence immunoassay (ECLIA) method.

After centrifugation, venous blood samples were stored at 80°C until the day of anti-Müllerian hormone (AMH) and copeptin

analyses. AMH analysis was performed using the ECLIA method (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). Copeptin levels were analyzed using an ELISA method (Human Vasopressin-Neurophysin 2-copeptin ELISA kit: EIAab Wuhan EIAab Science Co. Ltd.; East Lake Hi-Tech Development Zone, Wuhan 430079, China).

Sample Size Estimation and Matching Analysis

Priori power analysis was performed during the study planning; the test was used for the Stundent's t-test. The literature was reviewed, and the number of participants was estimated with the help of Cohen effect size[10]. In the analysis, a minimum of 35 participants were required for both the study and control groups for the 95% power hypothesis, 0.005 error, and 0.8 effect size. The R software was used to remove the age effect from the data (Version 1.2.5042, R Core Team, Vienna, Austria). The MatchIt library version 3.0.1 was used for case-control matching and for confounding due to age.

Statistical Analysis

Statistical analysis of the research data was performed using the SPSS software (Statistical Packages for Social Sciences, Chicago, IL, USA) version 21. The normality of the data distribution was analyzed using the Shapiro-Wilk test. The normal and non-normal data of the groups formed by the participants were examined with the Student's t-test and Mann-Whitney U test. After the analysis according to the statistical distribution, the data were presented in the tables as mean (± standard deviation) and median (minimum-maximum). The correlation between copeptin and the other parameters was examined using the Pearson correlation test. To determine the distinguishing power of the obtained data (sensitivity and specifity), receiver operating characteristic (ROC) analysis was performed, and graphs were drawn. The area under the curve in this figure was estimated with a 95% confidence interval. The Youdan index was used to determine the best intersection point in the ROC curve of the sensitivity and specificity values for the recognition of patients with POR. For the data to be significant, the p-value was set at <0.05.

Results

In total, 109 volunteers were included in our study; 52 of these volunteers were in the control group with a diagnosis of unexplained infertility, while 57 of them were participants with PCOS. No statistically significant difference was found between the demographic characteristics of the participants (p>0.05). The reproductive data and laboratory results are presented in Table 1. The AMH levels of the patients in the PCOS group were higher than those in the control group (p<0.001). Copeptin levels were higher in the control group (p<0.001).

Table 1. Comparison of the clinical and biochemical characteristics of the study parameters

	Control group (n=52) Mean ± std median (min-max)	PCO group (n=57) Mean ± std median (min-max)	p
Age (years)	31.1±4.3 31 (22-38)	29.9±5.1 30 (21-40)	0.321
BMI (kg/m^2)	22.1±2.5 21.8 (18.2-26.6)	22.7±2.4 22.2 (17.1-27.3)	0.412
Glucose (mg/dL)	81.2±12.5 83.0 (62.0-103.0)	83.2±9.7 81.0 (66.0-108.0)	0,745
Insuline (IU/mL)	13.6±3.3 (8.8-21.6)	12.8±2.5 (10.4-17.9)	0,195
HOMA IR	2.1±0.2 (1.0-2.3)	1.8±0.7 (15-2.4)	0.098
FSH (IU/L)	5.6±0.8 5.5 (4.2-8.3)	5.4±1.5 5.3 (2.4-9.6)	0.336
LH (IU/L)	5.1±0.9 5.2 (3.7-6.9)	5.9±1.3 5.8 (3.5-9.8)	**0.022***
AMH (ng/dL)	3.6±1.1 3.8 (1.7-5.8)	6.5±1.5 6.7 (3.3-9.7)	**<0.001***
AFC	13.0±4.1 13 (6-21)	14.8±4.8 15 (4-24)	0.111
Copeptin (ng/mL)	3.4±0.8 3.3 (2.7-5.3)	2.7±0.9 2.2 (1.9-6.6)	**<0.001***

BMI: Body mass index, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, AFC: Antral follice counts, Min: Minimum, Max: Maximum, Std: Standard

Correlation analysis was performed between copeptin and other reproductive parameters because the levels of copeptin were high in the control group and low in the PCOS group. A negative correlation was observed between Copeptin and AMH levels and LH levels (r=-227, p=0.047; r=-236, p=0.039, respectively), and no statistically significant correlation was found between other parameters and copeptin (p>0.05). Then, a new correlation analysis was performed between the reproductive parameters of the patients within the groups and their chronic periodontitis (CP) levels. In the correlation analysis performed in the control group, there was a negative correlation between copeptin and AMH (r=-0.402, p=0.013), while no statistically significant correlation was found between other reproductive parameters (p>0.05). In the correlation analysis performed within the PCOS group, a stronger negative correlation was found between AMH and copeptin levels (r=-0.544, p<0.01) also antral follice counts (AFC) and copeptin levels (r=-0.392, p=0.029). Correlation data within the PCO group are presented in Table 2.

ROC analysis of the CP levels was performed to determine PCOS predictivity. The estimated areas under ROC curves for serum concentration were found to be statistically significant (p<0.001) with a cut-off value of 2.78 (95% confidence interval 0.701-0.896), sensitivity of 0.87, and specificity of 0.70 (Table 3).

Discussion

This study aimed to determine the relationship between copeptin and reproductive function in patients with infertile PCOS. Copeptin levels were decreased in patients with PCOS without obesity and insulin resistance compared with healthy volunteers. The reproductive outcomes of patients with PCOS were negatively correlated with copeptin levels, and low copeptin levels had predictive value.

Copeptin levels are precursors of AVP and can be used as a stress marker in patients with PCOS. Because it is more stable in peripheral blood, biochemical measurements are considered more reliable. Copeptin is also used for diabetes insipidus and other diseases exhibiting vasopressin secretion changes. There is evidence that copeptin, like vasopressin, can be used in the follow-up of diabetes mellitus and respiratory and cardiovascular diseases[11,12]. Cardiovascular system diseases, diabetes mellitus, and obesity are frequently observed in PCOS patients[13].

Table 2. Correlation analysis of serum copeptin concentration and fertility parameters

	Copeptin control group (n=52)		Copeptin PCO (n=57)	
	r	p	r	p
Age (years)	0.252	0.327	-0.301	0.074
BMI (kg/m^2)	0.532	0.105	0.212	0.193
E2 (pg/mL)	0.224	0.466	0.351	0.091
FSH (IU/L)	0.481	0.179	0.377	0.038*
LH (IU/L)	0.195	0.281	0.401	0.052
AMH (ng/dL)	-0.480	0.033*	-0.544	<0.001*
AFC	-0.299	0.074	-0.392	0.029*

BMI: Body mass index, E2: Estradiol, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, AMH: anti-Müllerian hormone, AFC: Antral follice counts

Table 3. Serum low copeptin concentrations for predicting PCO development

Copeptin (ng/mL)	
AUC (95 % CI)	0.799 (0.701-0.896)
Cut-off	2.78
Sensitivity	0.87 (0.75-0.94)
Specificity	0.70 (0.66-0.88)
PPV	0.78 (0.65-0. 0.88)
NPV	0.88 (0.75- 0.95)
LR+	4.26 (2.55-7.12)
p	**<0.001**

AUC: Area under the curve, PPV: Positive predictive value NPV: Negative predictive value, LR: Learning rate

Stress markers and copeptin levels were elevated in patients with PCOS. Copeptin is believed to play an important role in the metabolic response and development of atherosclerosis in patients with insulin-resistant, hyperandrogenemic PCOS. It has been shown that copeptin levels are higher in patients with PCOS and accompanying increased carotid intima-media thickness, increased low-density lipoprotein levels, and insulin resistance compared with healthy volunteers[14]. Studies have shown that metabolic syndrome (MetS) and high Copeptin levels coexist, and PCOS patients have also been observed to have high copeptin levels in cases where MetS symptoms are present, and a correlation has been observed with insulin resistance, which is a component of MetS[15-17]. In our participants, fasting blood sugar levels were normal, insulin resistance was not present, and copeptin levels were lower than in the control group.

In our study, we found high AFC and AMH levels in the PCOS group, consistent with previous studies. We examined the relationship between copeptin levels and these two markers, which are markers of reproductive function, in both groups. An inverse correlation was observed between AMH and copeptin levels in both groups. It is known that inflammation is present in patients with PCOS and MetS, and interleukin (IL)-1 has a role in inflammation. Although copeptin levels are high in these patients, it has been shown that they do not change when IL-1 receptors are blocked in healthy volunteers and patients with PCOS who have MetS findings. In this case, the relationship between AMH and copeptin may develop through mechanisms other than hormonal changes[18].

Animal studies have also shown vasopressin neurons in the lamina terminalis (the area associated with sexual development) in the hypothalamic region. Because copeptin is produced from the c-terminus of pre-proAVP, the negative correlation with AMH in both groups may indicate changes in the hypothalamic region[19].

In our study, the relationship between AFC and copeptin was examined in both groups. An inverse correlation was found in the PCOS group, whereas no significant correlation was found in the control group. Because copeptin is known to be an osmodependent stress and inflammatory biomarker, the AFC count is expected to decrease during inflammatory and stress processes[20]. Although there were no MetS and proinflammatory stress findings in the PCOS group in our study, the negative correlation between AFC and copeptin may be attributed to changes in the follicular microenvironment.

Study Limitations

Although our study is specific because it evaluated patients with PCOS without MetS findings, it did not include follicle fluid analyses that can show hormonal and tissue-level changes that evaluate the hypothalamic-pituitary axis, such as corticotropin releasing hormone, AVP, and gonadotrophin-releasing hormone. Eliminating these limitations in future studies will increase the reliability of the data.

Conclusion

This study showed that low copeptin levels were lower in non-obese and insulin-resistant patients (without MetS symptoms) than in healthy volunteers. We showed that this finding was negatively correlated with AFC and AMH levels and that patients with PCOS with high copeptin levels (cut-off 2.7 ng/mL) may experience negative reproductive outcomes. Further studies are needed to investigate the effects of copeptin on tissue and follicular fluid levels in patients with PCOS who do not have MetS findings.

Ethics

Ethics Committee Approval: The research, which was designed as a case-control study, was carried out prospectively. Research ethics committee and approval were obtained from Hitit University Faculty of Medicine Clinical Research Ethics Committee (decision no: 383, date: 20.01.2021).

Informed Consent: Informed consent was obtained from all participants included in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ü.G., Concept: E.Y., Ü.G., Design: E.Y., Data Collection or Processing: E.Y., Analysis or Interpretation: E.Y., Literature Search: Ü.G., Writing: E.Y., Ü.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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