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The evaluation of serum Pancreatic-derived factor and Malondialdehyde levels in patients with polycystic ovary syndrome

Polikistik over sendrom'lu hastalarda Pancreatic Derived Factor ve Malondialdehit düzeylerinin incelenmesi

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Abstract

Purpose: Alterations in β-cell function may play crucial roles in the pathogenesis of polycystic ovary syndrome (PCOS). Pancreatic-derived factor (PANDER) is a cytokine-like protein, inducing of pancreatic β-cell apoptosis under pathological conditions. This investigation was planned to determine serum PANDER levels and establish whether serum PANDER levels are related with oxidative stress, and insulin resistance in PCOS.

Materials and methods: Twenty-seven patients with PCOS and 24 healthy control women were evaluated in this controlled clinical study. Serum lipid sub-fractions, fasting glucose, insulin, gonadotropins, androgens, malondialdehyde (MDA) and PANDER levels were measured. Homeostasis model assessment (HOMA-IR) were used to estimate insulin resistance.

Results: Subjects in study and control groups were similar with respect to waist measurements, gonadotropins, lipid sub-fractions, MDA and PANDER levels, the women with PCOS had considerably higher FAI and HOMA-IR than healthy women. Serum PANDER levels were not correlated with any studied parameters.

Conclusion: These outcomes showed that PANDER level is not related with insulin resistance, ovarian hyperandrogenism and oxidative stress in PCOS.

Key words: PCOS, PANDER, MDA, insulin resistance, oxidative stress.

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Özet

Amaç: β hücrelerinin fonksiyonlarındaki değişiklikler polikistik over sendromunun (PCOS) patogenezinde önemli roller oynayabilir. Pankreatic Derived Factor (PANDER), patolojik koşullar altında pankreatik β hücre apoptozunu indükleyen sitokin benzeri bir proteindir. Bu araştırma, serum PANDER düzeylerini belirlemek ve serum PANDER düzeylerinin oksidatif stres ve PCOS'taki insülin direnci ile ilişkili olup olmadığını belirlemek için planlanmıştır.

Gereç ve yöntem: Bu kontrollü klinik çalışmada PKOS tanısı almış 27 hasta ve 24 sağlıklı kadın kontrol grubu olarak değerlendirildi. Serum lipit alt fraksiyonları, açlık glikozu, insülin, gonadotropinler ve androjenler, malondialdehit (MDA) ve PANDER düzeyleri ölçüldü. İnsülin direncini tahmin etmek için homeostaz model değerlendirmesi (HOMA-IR) kullanıldı.

Bulgular: Çalışma ve kontrol grubundaki denekler bel ölçümleri, gonadotropinler, lipit alt fraksiyonları, MDA ve PANDER düzeyleri açısından benzerdi, PKOS'lu kadınlar sağlıklı kadınlardan önemli ölçüde daha yüksek FAI ve HOMA-IR'ye sahipti. Serum PANDER düzeyleri çalışılan parametrelerle ilişkili değildi.

Sonuç: Bu sonuçlar PANDER seviyesinin PCOS'ta insülin direnci, over kaynaklı hiperandrojenizm ve oksidatif stres ile ilişkili olmadığını göstermiştir.

Anahtar kelimeler: PCOS, PANDER, MDA, insülin direnci, oksidatif stres.

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Introduction

The polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women at reproductive age [1]. The diagnosis of PCOS is confirmed by international evidence-based guideline for the assessment and management of polycystic ovary syndrome including The Rotterdam criteria in women who have at least two of the following symptoms; ovarian dysfunction (oligo-amenorrhea), biochemical and/or clinical hyperandrogenism, enlarged polycystic ovaries [2, 3]. It has been suggested that insulin resistance, alterations in β-cell function and chronic low-grade inflammation may play pivotal roles in the pathogenesis of PCOS, though the absolute causal mechanisms has not been uncovered yet [4-7].

Pancreatic-derived factor (PANDER) is a cytokine-like protein, and it is expressed in the β-cells of the pancreas, the testis, the prostate, the small intestine, and the brain [8]. Also PANDER has a regulatory role in pancreatic β-cell function [9]. It was proposed that PANDER may be a potential activator of type 1 diabetes, because of inducing of pancreatic β-cell apoptosis [10]. However, the concept that PCOS is associated with alterations in β-cell function and PANDER is not well established. Furthermore, it can be considered that increased oxidative stress may cause β-cell dysfunction via PANDER in PCOS [11, 12].

Oxidative stress is emerged as a result of imbalance between the productions of free radicals and antioxidant defenses [13]. Increased oxidative stress is related to metabolic inflammation in several diseases such as diabetes and PCOS [4, 11-15]. Oxidative Stress induces apoptosis of pancreatic β-cells, because they are very susceptible to increased free radicals [16]. These data may lead to the following questions: whether there is an induced pancreatic β-cell apoptosis by PANDER in PCOS, and whether PANDER is related to oxidative stress in women with PCOS. During the last decade, changes in pancreatic β-cell function have gained attention to understand the mechanisms underlying anovulation in PCOS [17-19].

This investigation was planned to determine serum PANDER levels and establish whether serum PANDER levels are related to oxidative stress, ovarian hyperandrogenism, lipid fractions, and insulin resistance in women with PCOS. Oxidative stress was evaluated by the levels of malondialdehyde (MDA). To the best of our knowledge, we present the first study regarding serum PANDER levels in PCOS.

Materials and methods

Subjects

We studied twenty-seven patients with PCOS (study group) and 24 healthy women (control group). The patients' ages ranged from 17 to 41 years. This study was permitted by local medical ethics committee and the informed consent forms were signed by every participants at the beginning of investigation.

Medical history, physical and pelvic examinations, and all blood chemistry were performed to evaluate the health status of subjects. PCOS was diagnosed by criteria of The Androgen Excess Society (AES) by the following features: 1-biochemical and/or clinical hyperandrogenism, and 2-Ovarian dysfunction: oligo-anovulation and/or polycystic ovaries, and 3-Exclusion of other androgen excess or related disorders [20]. The women in the control group had regular menstrual cycles (cyclic uterine bleedings with duration of 4-5 days and a frequency of 25–34 days/month).

Exclusion criteria included diabetes, thyroid dysfunction, hyperprolactinemia, Hyperandrogenic-Insulin Resistance-Acanthosis Nigricans syndrome, androgen secreting tumors, late-onset 21-hydroxylase deficiency, Cushing's syndrome, the, family history of cardiovascular disease, hypertension, infectious diseases, use of androgenic/anabolic drugs or medications known to alter insulin and lipoprotein metabolism, consuming alcohol and/or smoking. None of participants met any exclusion criteria.

Ethics committee approval has taken in Pamukkale University Non Interventional Clinical Researches Ethics Committee.

Biochemical analysis

Blood samples were taken after 10-hour fasting on the study day (on cycle, days 3-5 after spontaneous or progesterone-induced menses). Serum fasting glucose (F. Glc),

triglyceride (TG), and total cholesterol (TC) were obtained using standard enzymatic methods (Roche Diagnostics, IN, US) with a fully automated analyzer (Roche Modular PE, Roche Diagnostics, IN, US). High-density lipoprotein cholesterol (HDL-C) levels were determined using liquid selective detergent homogeneous technique (Roche HDL-C plus 2nd generation, Roche Diagnostics, IN, US). Low-density lipoprotein cholesterol (LDL-C) levels were calculated by Friedewald's formula.

Fasting insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone concentrations were measured using electrochemiluminescence's immunoassay (Roche Diagnostics, IN, US) with a fully automated analyzer (Roche Modular PE, Roche Diagnostics, IN, US). Sex hormone-binding globulin (SHBG) and dehydroepiandrosterone sulphate (DHEAS) measurements were performed using a solid phase competitive chemiluminescence immunoassay (IMMULITE 2000, DPC Biosystems, CA, USA).

Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR, the formula: fasting insulin concentration (mIU/l) x glucose (mmol/l)/22.5) [21], and Individuals with HOMA-IR > 2.7 were accepted as insulin resistant [22, 23]. Free androgen index (FAI) was defined as 100 times the molar ratio of total testosterone to SHBG [FAI = 100 X total testosterone (nmol/l) / SHBG (nmol/l)].

PANDER was measured using commercially available enzyme-linke immunosorbent assay (ELISA) test kits (Cloud-Clone Corp.). The sensitivity is 0.255 ng/ml, and the detection range is 0.625 ng/ml to 40 ng/ml. All samples were tested in triplicate. For biochemical measurements, the within-run coefficients of variability (CV) and between-run CV values were <10% and <12% respectively.

The serum MDA levels were determined by the procedure of Ohkawa et al. [24]. 0.5 ml of serum was mixed with 1.5 ml thiobarbituric acid (0.8%), 1.5 ml acetic acid (pH 3.5, 20%), 0.2 ml sodium dodecyl sulfate (8.1%) and 0.5 ml distilled water. After mixing, all samples and standards were heated at 100°C for one hour. The absorbance was recorded at 532 nm and compared with those of MDA standards.

Anthropometric measurements

All anthropometric measurements were performed by the same physician on the day blood specimen were taken. Waist and hip circumferences (cm) were obtained and body mass index (BMI) (Body weight (kg) / height m^2) and waist-to-hip ratio (WHR) were computed.

Statistical analysis

At the beginning of the study, all study participants were matched for age and BMI. The healthy controls were defined as age- and BMI-matched with subjects when the number of year's ± age of subjects and the BMI of subjects were less than to 2 years and less than to 1 kg/m^2, respectively. Data were analyzed by using the SPSS (Statistical Package for the Social Science, version 17.0). The data are expressed as means ± SE (standard error). Since many variables had a gaussian distribution with significant skewness, statistical analysis was performed by using a parametric test: Student's t-test. Correlations between variables were calculated by using Pearson's correlation coefficient. All P values presented are two-tailed; $p<0.05$ was considered statistically significant.

Results

Subjects in study and control groups were similar with respect to waist measurements, total cholesterol, triglyceride, HDL-C, LDL-C, FSH, DHEAS, MDA and PANDER levels. We found significant differences in total testosterone, SHBG, FAI, LH levels, LH/FSH ratio, HOMA-IR, serum fasting glucose, and insulin levels between study and control groups (Table 1 and 2).

We did not detect any significant correlations between PANDER and the other parameters. However, FAI was positively correlated with HOMA-IR (r=0.50, p=0.0001) and TG (r=0.51, p=0.0001), and BMI (r=0.57, p=0.0001), inversely with HDL-C (r=-0.38, p=0.006). In addition, waist measurement was positively related to total cholesterol (r=0.32, p=0.021), TG (r=0.33, p=0.018), and LDL-C (r=0.43, p=0.002). HOMA-IR was also positively associated with TG (r=0.35, p=0.013), but negatively related to HDL-C (r=-0.30, p=0.033). Also HDL-C was inversely correlated with LDL-C (r=-0.32, p=0.016) and TG (r=-0.36, p=0.009).

Table 1. Clinical features and steroid levels for both the women with PCOS and the healthy controls

Variable	Women with PCOS (n=27)	Healthy Controls (n=24)	*p*
Age (years)	23.3± 0.6	25.1± 0.9	0.10
BMI (kg/m^2)	24.9± 1.3	22.3± 0.9	0.11
Waist (cm)	79.0± 3.0	74.8± 2.5	0.29
FSH (mIU/ml)	6.1± 0.3	6.4± 0.3	0.59
LH (mIU/ml)	8.9± 1.0	6.3± 0.4	0.03[a]
LH/FSH ratio	1.5± 0.2	1.0± 0.1	0.02[a]
Total testosterone (ng/mL)	0.5±0.03	0.3± 0.03	0.008[a]
SHBG (nmol/L)	40.6± 4.1	55.8±5.3	0.03[a]
DHEAS (μg/dl)	289.7±24.5	215.7±16.1	0.90
FAI	5.2± 0.8	2.5± 0.5	0.008[a]

[a] $p<0.05$ statistically significant. BMI: Body Mass Index, FSH: Follicle-Stimulating Hormone, LH: Luteinizing Hormone, DHEAS: Dehydroepiandrosterone Sulphate, SHBG: Sex Hormone-Binding Globulin, FAI: Free Androgen Index

Table 2. Metabolic characteristics, Malondialdehyde, PANDER levels for both the women with PCOS and the healthy controls

Variable	Women with PCOS (n=27)	Healthy Controls (n=24)	*p*
Fasting Insulin (μIU/mL)	12.8±1.5	7.3±0.6	0.002[a]
Fasting glucose (mg/dL)	84.1±1.8	78.9±2.4	0.04[a]
HOMA-IR	2.7±0.3	1.4±0.1	0.001[a]
Total cholesterol (mg/dL)	160.3±5.6	168.1±5.4	0.28
Triglyceride (mg/dL)	82.5±8.4	86.4±10.4	0.77
HDL-C (mg/dL)	54.5±3.0	59.2±2.0	0.93
LDL-C (mg/dL)	91.9±5.5	92.6±4.4	0.93
MDA (nmol/mL)	10.2±0.5	11.0±0.7	0.37
PANDER (ng/mL)	1.2± 0.1	1.3± 0.1	0.44

[a] $p<0.05$ statistically significant. HOMA-IR: Homeostasis Model Assessment, QUICKI: Quantitative Insulin Sensitivity check Index, HDL-C: High-Density Lipoprotein-Cholesterol, LDL-C: Low-Density Lipoprotein-Cholesterol, MDA: Malondialdehyde, PANDER: Pancreatic-Derived Factor

As expected, TG was positively associated with total cholesterol (r=0.52, p=0.0001), and LDL-C (r=0.52, p=0.0001).

Discussion

The results of our study showed that PANDER levels were slightly higher in control subjects than in studies, although it was statistically insignificant. It was suggested that PANDER might be responsible for several roles in glucose homeostasis under physiological conditions, even though PANDER has a potential role in pancreatic islet apoptosis under pathological conditions [25-28]. Both glucose and insulin have important effects on the regulation of PANDER [29-31]. Indeed, the insulin resistance and hyperglycemia induce expression of PANDER [27]. Furthermore, PANDER may be related to low-grade inflammation as a pro-inflammatory cytokines of pancreatic islets [32, 33]. Remarkably, a considerable proportion of women with

PCOS has insulin resistance and low-grade chronic inflammation [11-13]. Therefore, the question is whether PANDER has a role in the pathogenesis of PCOS.

There was no correlation between PANDER and the other study parameters. Hence, our study suggests that PANDER is not responsible for inducing diabetic process through β-cell dysfunction in PCOS. The association between

PANDER and oxidative stress, and insulin resistance may not be as stronger as we thought in women with PCOS. Studies suggest that elevation of PANDER is more clearly noticeable in the worsening stages of Type 2 DM, which might be related partially to an advanced degree of beta-cell dysfunction [12]. This finding may be the reason for the indifference of PANDER levels in our study groups who don't have overt diabetes.

Oxidative stress is emerged as a result of imbalance between the productions of free radicals and antioxidant defenses [14]. Increased oxidative stress is related to metabolic inflammation in several diseases such as diabetes and PCOS [4, 11-17]. Also, oxidative stress aggravates apoptosis of pancreatic β-cells, because they are very susceptible to increased free radicals [18]. The extent and nature of oxidative stress could not directly be measured in biological systems, therefore a vast number of biomarkers such as MDA have been identified and used to determine oxidative damage [32]. In this study, interestingly, we did not find significant differences in serum MDA levels between the groups. However, young age of women in this study may explain the steady state levels of MDA.

We did not observe any considerable differences in serum lipid fractions in PCOS women, compared with matched-for-age and BMI control subjects. However, dyslipidemia can be detected in oxidative stress conditions [33]. There are positive relationships between hyperinsulinemia and the ovarian hyperandrogenism [34]. Nevertheless, this observation was not completely confirmed by our results. In fact, there was not a correlation between FAI and HOMA-IR, and PANDER. On the other hand, FAI was positively associated with FAI was positively correlated with HOMA-IR, TG, and BMI, while inversely with HDL-C. In addition, there was positive relationship between waist measurement and total cholesterol, TG, and LDL-C. Furthermore, HOMA-IR was also positively associated with TG, but negatively related to HDL-C. The few numbers of the subjects in this study may be a reason. Another logical explanation is that the lack of an important alteration in oxidative stress may mask our results.

In conclusion, results of current investigation showed that serum PANDER level is not related to insulin resistance, ovarian hyperandrogenism and oxidative stress in patients with PCOS. But, alterations in β-cell function in the pathogenesis of PCOS remains to be elucidated.

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

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Contributions of the authors to the article

Ü.Ç. and B.K. constructed the main idea and hypothesis of study. Ü.Ç, B.K and İ.V.F. developed the theory and organized the material method section. İ.K and S.D. made the evaluation of data in results section. Discussion section of the article written by Ü.Ç., B.K and İ.V.F. has reviewed and made the necessary corrections and approved. In addition, all authors discussed the entire study and confirmed its final version.