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Lipocalin-2 is not related with insulin resistance and chronic low-grade inflammation in women with polycystic ovary sindrome

Soner Gok[1], Umit Cabus[2], Ilyas Turan[2], Cihan Kabukcu[2], Veysel Fenkci[2], Nedim Karagenc[3]

[1] Departments of Obstetrics and Gynecology, Gynecology and Children's Sate Hospital, Aydin, Turkey
[2] Departments of Obstetrics and Gynecology, Pamukkale University, School of Medicine, Denizli, Turkey
[3] Department of Medical Biology, Pamukkale University, School of Medicine, 20100 Denizli, Turkey

ABSTRACT

Background: polycystic ovary syndrome (PCOS) may be related to a low-grade chronic inflammation, via insulin resistance. Lipocalin-2 is an inflammatory marker. This study was designed to determine serum lipocalin-2 levels and establish whether serum lipocalin-2 levels are related with oxidative stress, insulin resistance, ovarian hyperandrogenism, and dyslipidemia in women with PCOS.

Methods: twenty-four patients with PCOS and 27 healthy control women were evaluated in this controlled clinical study. Serum lipid sub-fractions, fasting glucose, insulin, gonadotropins, androgens, malondialdehyde (MDA) and lipocalin-2 levels were measured. Homeostasis model assessment (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were used to estimate insulin resistance.

Results: free androgen index (FAI), total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) levels were significantly higher in subjects with PCOS. Serum lipocalin-2 levels were slightly higher in study subjects than in controls, although it was statistically insignificant. Serum lipocalins-2 levels were not correlated with any studied parameters.

Conclusions: these outcomes propose that lipocalin-2 is not associated with insulin resistance and may not play an essential role in chronic low-grade inflammation of PCOS, although the absence of central obesity in women in this study may mask the relationship between lipocalin-2 and insulin resistance.

Key words: PCOS, Lipocalin-2, Oxidative stress, Insulin Resistance

Corresponding Author: Veysel Fenkci
veyselfenkci@yahoo.com

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SOMMARIO

Introduzione: la sindrome dell'ovaio policistico (PCOS) può essere correlata ad una infiammazione cronica di basso grado attraverso la resistenza all'insulina. Lipocalin-2 è un marcatore infiammatorio. Questo studio è stato realizzato per determinare i livelli di siero lipocalina-2 e stabilire se i livelli di siero lipocalina-2 sono correlati con lo stress ossidativo, la resistenza all'insulina, l'iperandrogenismo ovarico e la dislipidemia nelle donne con PCOS.

Metodi: in questo studio clinico controllato sono state valutate ventiquattro pazienti con PCOS e 27 donne sane. Sono state misurate le sottosezioni lipidiche del sangue, il glucosio a digiuno, l'insulina, le gonadotropine, gli androgeni, i malondialdehidi (MDA) e i livelli di lipocalin-2. La valutazione del modello di homeostasi (HOMA-IR) e l'indice di controllo quantitativo di sensibilità all'insulina (QUICKI) sono stati utilizzati per stimare la resistenza all'insulina.

Risultati: l'indice di androgeno libero (FAI), il colesterolo totale (TC), il trigliceride (TG) ei livelli di colesterolo lipoproteico a bassa densità (LDL-C) erano significativamente più alti nei soggetti con PCOS. I livelli di siero lipocalina-2 erano leggermente superiori nei soggetti in studio rispetto ai controlli, anche se erano statisticamente insignificanti. I livelli di siero di lipocalin-2 non erano correlati con alcun parametro studiato.

Conclusioni: questi risultati suggeriscono che il lipocalin-2 non sia associato alla resistenza all'insulina e non può svolgere un ruolo essenziale nell'infiammazione cronica di basso livello di PCOS, anche se l'assenza di obesità centrale nelle donne in questo studio può mascherare la relazione tra lipocalin-2 e la resistenza all'insulina.

Parole chiave: PCOS, Lipocalin-2, Sforzo ossidativo, Resistenza all'insulina

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder, affecting approximately 7% of women at reproductive age [1], and it is characterized by oligo-amenorrhea, enlarged cystic ovaries, signs of androgen overproduction, disordered gonadotropin secretion, and reduced fertility [2]. Insulin resistance (IR) plays pivotal roles in the pathogenesis of PCOS, even if the mechanisms underlying PCOS are not completely understood [3,4]. The association between disorders of lipid metabolism and insulin resistance is also obvious.

Lipocalin-2 (also known as neutrophil gelatinase-associated lipocalin) is an inflammatory marker [5]. It was initially identified in human neutrophil granules, but also lipocalin-2 is expressed in numerous other tissues, such as liver, kidney, lung, adipocytes, and macrophages [6,7]. PCOS may be related to a low-grade chronic inflammation, most probably through the development of insulin resistance [8]. Interestingly, this secretory glycoprotein is closely related with obesity and insulin resistance and has a role in apoptosis and innate immunity [9]. However, its exact physiological importance in insulin signaling and chronic inflammatory process in women with PCOS is still unclear.

Oxidative stress is an imbalance between the productions of free radicals and antioxidant status [10]. It is considered to be one of the main causes of molecular damage to cellular and tissue structures [10,11]. Oxidative stress is also known to be increased in the pathogenesis of several diseases such as diabetes and PCOS [11,12]. All of the aforesaid data can address the question as to whether there is a relationship between insulin resistance and serum Lipocalin-2 level in PCOS, and whether Lipocalin-2 is associated with oxidative stress in women with PCOS.

This study was designed to determine serum Lipocalin-2 levels and establish whether serum Lipocalin-2 levels are linked with insulin resistance, oxidative stress, ovarian hyperandrogenism, and dyslipidemia in women with PCOS. Oxidative stress was evaluated by the levels of malondialdehyde (MDA).

MATERIALS AND METHODS

Subjects

Twenty-four patients with PCOS (study group) aged between 17-39 years and 27 healthy women (control group) aged between 19-37 years were included in the study. Health status of subjects was determined by medical history, physical and pelvic examinations, and all blood chemistry. This investigation was approved by local medical ethics committee and all participants gave informed consent before the beginning of study.

The diagnosis of PCOS was based on The Androgen Excess Society (AES) criteria on PCOS by the following features: 1- Hyperandrogenism: hirsutism and/or hyperandrogenemia, and 2- Ovarian dysfunction: oligo-anovulation and/or polycystic ovaries, and 3- Exclusion of other androgen excess or related disorders [13]. Common findings in PCOS group were; oligomenorrhea (≤6 menses/year), 12 or more subcapsular follicles by transvaginal ultrasonography, clinical hyperandrogenism with the presence of acne and hirsutism (Ferriman-Gallwey score of ≥ 8) [14]. Exclusion criteria included thyroid dysfunction, hyperprolactinemia, androgen secreting tumors, late-onset 21-hydroxylase deficiency, diabetes, Cushing's syndrome, the Hyperandrogenic-Insulin Resistance-Acanthosis Nigricans syndrome, 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.

The subjects 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) and none of them met any exclusion criteria.

Biochemical analysis

Venous samples were drawn in the morning after at least 10 hours of fasting on the study day (on cycle, days 3-5 after spontaneous or progesterone-induced menses). The sera were assayed for fasting glucose (F.Glc), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), insulin, dehydroepiandrosterone sulphate (DHEAS), sex hormone-binding globulin (SHBG), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, MDA. Serum glucose, total cholesterol and triglyceride concentrations were measured using standard enzymatic methods (Roche Diagnostics, IN, US) with a fully automated analyzer (Roche Modular PE, Roche Diagnostics, IN, US). HDL-C concentrations were measured without precipitation by 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, FSH, 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). SHBG, 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) [15], and the quantitative insulin sensitivity check index (QUICKI, the formula: 1/log(fasting glucose (mg/dl)) + log(fasting insulin (mIU/l)) [16]. 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)].

Lipocalin-2 was measured using commercially available enzyme-linke immunosorbent assay (ELISA) test kits (BioVendor R&D Products). The sensitivity is 0.02 ng/ml, and the detection range is 0.02 ng/ml to 100 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. [17]. 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 done by the same physician on the day blood specimen were taken. Body mass index (BMI) (Body weight (kg) / height m2) was 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/m2, respectively. Statistical analysis was performed with a parametric test: Student's t-test. Correlations between variables were calculated with Pearson's correlation coefficient. The data are expressed as means ± SE (standard error). All P values presented are two-tailed; $P < 0.05$ was considered statistically significant. Data were analyzed with the SPSS (Statistical Package for the Social Science, version 17.0).

RESULTS

There were no statistically significant differences in waist measurement, HOMA-IR, QUICKI, serum fasting glucose, insulin, SHBG, DHEAS, HDL-C, MDA and lipocalin-2 levels between the groups. The women with PCOS had considerably higher hirsutism scoring, LH/FSH ratio, FAI, serum LH, total testosterone, total cholesterol, triglyceride, and LDL-C levels than healthy women, while serum FSH levels were significantly lower in patients with PCOS compared with controls (**Table I and II**).

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

Variable	Women with PCOS (n = 24)	Healthy Controls (n = 27)	p
Age (years)	22.1 ± 0.9	24.2 ± 0.9	0.12
BMI (kg/m^2)	22.9 ± 0.9	22.3 ± 0.6	0.55
Waist (cm)	81.9± 3.4	77.5 ± 1.9	0.27
Hirsutism Scoring	10.2 ± 0.2	4.3 ± 0.1	0.0001[a]
FSH (mIU/ml)	5.6 ± 0.2	6.6 ± 0.4	0.04[a]
LH (mIU/ml)	9.7 ± 0.8	5.7 ± 0.3	0.0001[a]
LH/FSH ratio	1.8 ± 0.2	0.9 ± 0.1	0.0001[a]
DHEAS (µg/dl)	291.8 ± 21.7	246.7 ± 15.8	0.10
Total testosterone (ng/ml)	0.4 ± 0.03	0.3 ± 0.02	0.013[a]
SHBG (nmol/l)	25.3 ± 3.5	35.2 ± 3.6	0.054
FAI	7.8 ± 1.3	3.7 ± 0.5	0.007[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, Lipocalin-2 levels for both the women with PCOS and the healthy controls.

Variable	Women with PCOS (n = 24)	Healthy Controls (n = 27)	p
Fasting Insulin (µIU/ml)	11.0 ± 1.2	8.7± 0.9	0.14
Fasting glucose (mg/dl)	88.1 ± 2.6	88.2 ± 2.0	0.98
HOMA-IR	2.5 ± 0.3	1.9 ± 0.2	0.16
QUICKI	0.35 ± 0.06	0.36 ± 0.04	0.78
Total cholesterol (mg/dl)	173.7 ± 5.4	148.7 ± 4.7	0.001[a]
Triglyceride (mg/dl)	97.5 ± 10.9	61.6 ± 5.2	0.005[a]
HDL-C (mg/dl)	55.0 ± 3.1	59.7 ± 2.8	0.27
LDL-C (mg/dl)	101.0 ± 6.2	77.1 ± 4.0	0.002[a]
MDA (nmol/ml)	2.5 ± 0.2	2.7 ± 0.3	0.64
Lipocalin-2 (ng/ml)	277.0 ± 5.7	272.5 ± 6.3	0.59

[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

Waist measurement was positively related with BMI (r=0.84, p=0.0001), HOMA-IR (r=0.41, p=0.003), total cholesterol (r=0.41, p=0.003), triglyceride (r=0.37, p=0.008), and LDL-C (r=0.43, p=0.002). Hirsutism scoring was also positively associated with FAI (r=0.50, p=0.0001), LH/FSH ratio (r=0.59, p=0.0001), waist measurement (r=0.30, p=0.035), total cholesterol (r=0.46, p=0.001), triglyceride (r=0.50, p=0.0001), and LDL-C (r=0.46, p=0.001). FAI was positively correlated with triglyceride (r=0.60, p=0.0001) and HOMA-IR (r=0.34, p=0.007), but inversely with HDL-C (r= -0.45, p=0.001). HOMA-IR was positively linked with triglyceride (r=0.43, p=0.001). There was a positive relationship between total cholesterol, triglyceride (r=0.45, p=0.001), and LDL-C (r=0.87, p=0.0001) levels. In addition, triglyceride was positively related with LDL-C (r=0.42, p=0.002).

DISCUSSION

Lipocalin-2 belonging to the superfamily of lipocalins has an important role in transporting lipophilic molecules and in innate immunity [18]. It have recently shown that lipocalin-2 secretion is associated with central obesity, insulin resistance, and low-grade inflammation [19,20]. Since a substantial proportion of women with PCOS has insulin resistance and low-grade chronic inflammation, it is reasonable to presume that lipocalins-2 action may contribute to the development of insulin resistance in PCOS. Our results did not support this suggestion; actually, serum lipocalin-2 levels were slightly higher in study subjects than in controls, although it was statistically insignificant. In addition, serum lipocalins-2 levels were not correlated with any studied parameters. Interestingly, these outcomes were partially consistent with the observations of some previous reports [21,22]. These outcomes propose that lipocalin-2 may not play an essential role in the pathogenesis of PCOS. Possible limitation of this study includes that the absence of central obesity in women in this study may mask the relationship between lipocalin-2 and insulin resistance. Additionally, the few numbers of the subjects in this study may be a reason.

Oxidative stress is considered to be one of the major causes of several pathological disorders, particularly in terms of molecular damage to cellular structures [23]. It also aggravates the symptoms of numerous diseases. It was previously shown that there was increased oxidative stress and decreased antioxidant capacity in women with PCOS (12). Several biomarkers such as MDA has been identified to determine oxidative damage to biomolecules, although it is not always possible to measure directly in biological systems [24]. In this study, there were no significant differences in serum MDA levels between the groups. In addition, it was not associated with other parameters. It will be logical to assume that the absence of a significant change in HOMA-IR may explain the steady state levels of MDA.

Dyslipidemia is often accompanied by reduced HDL, and elevated LDL, total cholesterol, triglyceride levels. It can be observed in insulin resistance and increased oxidative stress circumstances [25]. In current investigation, higher total and LDL cholesterol levels, and higher triglyceride levels were more common in our PCOS subjects, compared with matched-for-age and BMI control women, as have been shown in previous studies [26,27]. Furthermore, serum lipid fractions were positively related with hyperandrogenism and insulin resistance. In fact, androgen overproduction was positively associated with total cholesterol, triglyceride, and LDL-C, but inversely with HDL-C. It has been known that there is a positive relationship between hyperinsulinemia and the ovarian hyperandrogenism [28]. This observation was also confirmed by our results. FAI was positively correlated with HOMA-IR. Although increased clinical and biochemical signs of hyperandrogenism were observed in women with PCOS, insulin resistance was not significantly changed in our investigation. The selection of the subjects, who had normal BMI, might be a reason. Briefly, these outcomes revealed that ovarian hyperandrogenism was a stronger predictor for dyslipidemia than for insulin resistance at least in this study. Interestingly, we did not observe any association between lipid fractions and serum lipocalins-2 level. The relationship between lipocalins-2 and dyslipidemia may not be as stronger as we thought in women with PCOS.

In conclusion, although the outcomes of this study suggest that lipocalin-2 level is not associated with insulin resistance, ovarian hyperandrogenism and oxidative stress, the pivotal role in triggering the processes leading to chronic low-grade inflammation in the pathogenesis of PCOS remains to be elucidated. In terms of lipocalin-2 activity, long-term studies are needed in PCOS, especially in molecular basis.

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