

The relationship between biochemical parameters, interleukin-6 and ovarian morphology in polycystic ovary syndrome

Polikistik over sendromunda biyokimyasal parametreler, interlökin-6 ve over morfolojisi arasındaki ilişki

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ABSTRACT

Objectives: The aim of this study is to investigate relationships between biochemical parameters and interleukin-6 according to the ovarian morphology in polycystic ovary syndrome (PCOS).

Materials and methods: Total of 85 patients with PCOS were divided into three groups according to the ultrasonographic examination of the ovaries; group 1 (with no polycystic image at any of the ovaries, n=22), group 2 (with polycystic image at only one ovary, n=41), and group 3 (with polycystic image at both of the ovaries, n=22). Serum interleukin 6 (IL-6), hormone levels, and homeostasis model assessment-insulin resistance (HOMA-IR) were measured.

Results: The mean serum IL-6 levels for groups 1, 2 and 3 were 14.65 ± 6.18 pg/mL, 16.22 ± 6.55 pg/mL, and 14.53 ± 7.32 pg/mL, respectively. There was no statically difference between three groups for serum IL-6 levels ($p>0.05$). For metabolic profile, the mean of HOMA-IR was 3.09 ± 1.56 for group 2 and this was significantly lower than other groups ($p<0.05$).

Conclusions: There were no difference in hormonal and inflammatory markers according to the ovarian morphology in PCOS patients based on data derived from our study. *J Clin Exp Invest* 2012; 3(3): 307-312

Key words: PCOS, interleukin-6, ovarian morphology.

ÖZET

Amaç: Bu çalışmanın amacı polikistik over sendromunda (PKOS) biyokimyasal parametreler ve interleukin-6 ile over morfolojisi ilişkisini değerlendirmektir.

Gereç ve yöntem: PKOS tanısı almış 85 hasta overlerin ultrasonografik değerlendirmesine göre; grup 1 (polikistik görünümü olmayan, n = 22), grup 2 (bir overde polikistik görüntüsü olan, n = 41) ve grup 3 (her iki overde polikistik görüntüsü olan, n = 22) olmak üzere üç gruba ayrıldı. Hastaların serum interlökin 6 (IL-6), hormone düzeyleri ve homeostasis model assessment-insulin rezistans (HOMA-IR) değerleri ölçüldü.

Bulgular: Serum IL-6 seviyeleri değerlendirildiğinde, ortalama değerler gruplar için sırasıyla 14.65 ± 6.18 pg/mL, 16.22 ± 6.55 pg/mL ve 14.53 ± 7.32 pg/mL olarak bulundu. Serum IL-6 düzeyleri için üç grup arasında istatistiksel olarak anlamlı bir fark yoktu ($p> 0.05$). Metabolik profilin değerlendirilmesinde ise HOMA-IR değeri grup 2 de 3.09 ± 1.56 bulundu ve diğer gruplara göre anlamlı düzeyde düşüktü ($p<0.05$).

Sonuç: Çalışmamız verilerine dayanarak, PKOS hastaları için over morfolojisine göre hormonal ve inflamatuvar belirteçler arasında fark yoktu.

Anahtar kelimeler: PKOS, interlökin-6, over morfolojisi.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting about 6% of women of reproductive age which is characterized by both gynecological and endocrine symptoms, including chronic anovulation and hyperandrogenism.¹ In 2003, the European Society for Human Reproduc-

tion and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) revised the definition of PCOS 1; the syndrome is now defined as the presence of any two of the following three criteria: polycystic ovaries, oligo/anovulation and/or clinical or biochemical evidence of hyperandrogenism.¹ In addition to the reproductive abnor-

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malities, a significant proportion of PCOS women suffer from obesity, insulin resistance and features of the metabolic syndrome.² Other factors that independently increase cardiovascular risk, such as obesity, arterial hypertension, dyslipidaemia and clotting disorders, may coexist in women with PCOS.^{3,4}

Inflammatory processes, particularly increased proinflammatory cytokine production and activation of immune system, are associated with obesity, and have been implicated in the pathophysiology of atherosclerosis, the metabolic syndrome, insulin resistance, and diabetes mellitus.⁵ There is accumulating evidence indicating chronic low-grade inflammation in PCOS, including increased levels of C-reactive protein (CRP), increased leukocyte numbers, and increased pro-inflammatory cytokines.⁶ Human interleukin 6 (IL-6) is a 184 AA polypeptide with potential O- and N-glycosylation sites and a significant homology with G-CSF. It is produced by various cells, including T and B cells, monocytes, fibroblasts, endothelial cells, mesangial cells and several tumor cells. IL-6 regulates the growth and differentiation of various cell types with major activities on the immune system, hematopoiesis and inflammation.⁷ The elevation of serum IL-6 precedes that of acute phase proteins, e.g., in a postoperative phenomenon and may thus be a sensitive early parameter to investigate inflammatory conditions. IL-6 signaling molecule is associated with hyperandrogenism and PCOS, or influence hyperandrogenic phenotypic traits.⁸ The adipose tissue plays common role in the relationship between cytokines and insulin resistance. Inflammatory cytokines might induce insulin resistance by direct actions on insulin-signaling post receptor molecules or by inducing central obesity through activation of the hypothalamic-pituitary-adrenal axis.⁹

Polycystic ovary morphology is a cardinal feature of PCOS defined as irregular menses and hyperandrogenism.^{10,11} But the presence of polycystic ovary morphology does not enough for diagnosis of PCOS and may be seen with normal women.^{1,12} On the other hand there is not adequate information about the characteristics of inflammation marker of PCOS patients with normal ovarian morphology. The aim of this study is to estimate relationship with the biochemical parameters and interleukin-6 according to the ovarian morphology in PCOS.

MATERIALS AND METHODS

Study subjects

Total of 85 non-smoking subjects with PCOS who were admitted to the department of gynecology and

obstetrics of Faculty of Medicine of Kirikkale University were enrolled in this study. They were divided according to the ultrasonographic examination of the ovaries into three groups; group 1 (with no polycystic image at any of the ovaries), group 2 (with polycystic image at only one ovary), and group 3 (with polycystic image at both of the ovaries). All women were euthyroid and not taking any medication or hormones for six months (including oral contraceptive agents) known to affect sex hormones or carbohydrate metabolism. The diagnosis of PCOS was made on the basis of the revised 2003 Rotterdam ESHRE/ASRM diagnostic criteria¹, women with PCOS meet two of the following three criteria after exclusion of other etiologies (pituitary insufficiency, persistent hyperprolactinemia, congenital adrenal hyperplasia): (a) oligomenorrhea or anovulation; (b) clinical and/or biochemical signs of hyperandrogenism; (c) polycystic ovaries.

Age, height, body weight, waist circumference and systolic and diastolic blood pressure were recorded. Waist circumference was taken midway between the inferior margin of the last rib and the crest of the ileum in the horizontal plane, while in an upright position. Body mass index (BMI) was calculated for all groups as body weight (kg) divided by body height squared (m²). Waist/Hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Ferriman-Gallwey score ≥ 7 was used for grading hirsutism.¹³

Radiologic procedures

Ultrasonographic examinations were performed in the early follicular phase (days 1 ± 3) of the menstrual cycle, when the ovaries are relatively quiescent by a single operator following the criteria of the Rotterdam ESHRE/ASRM Consensus.¹ Transabdominal ultrasonographic examination was performed on each patient by using linear-array real time ultrasound with a 2.0-MHz transducer (GE LOGIQ 9 Milwaukee, USA).

Sample collection

Blood was drawn for determination of basal endocrinological and immunological parameters between 9:00 and 10:00. Fasting blood samples were taken from the antecubital vein at rest in the supine position at the morning. Blood was immediately transferred into ice-chilled glass tubes containing disodium EDTA (1 mg/ml) and centrifuged 1600 g, for 15 min at 4°C. Serum samples were frozen and stored at -70°C until assayed.

Biochemical procedures

The fasting blood sample was analyzed for serum thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), androstenedione (AS), dehydroepiandrosterone-sulphate (DHEAS), total testosterone (Ttes) and free testosterone (Ftes) on day 2 or 3 of menstrual cycle while serum progesterone was measured on day 21.

Serum IL-6 levels were measured by ELISA (Human IL-6 Immunoassay Kit, KHC0061, Bio-Source International, USA) as described previously.¹⁴

After 12 h of fasting, serum glucose, total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations were determined using an autoanalyzer into the standard laboratory measurements. Serum insulin was measured by immunoradiometric assay. The estimate of insulin resistance by the homeostasis model assessment (HOMA-IR) was calculated with the following formula; fasting serum insulin ($\mu\text{IU/mL}$) \times fasting serum glucose (mmol/L) \div 22, 5.¹⁵ The HOMA-IR value was given as mean \pm SD, and appraised the values $> 2,5$ as an insulin resistant state and the values $< 2,5$ as an insulin sensitive state.

Glucose tolerance was evaluated using 75g Oral Glucose Tolerance Test (OGTT). Abnormal plasma OGTT was classified according to the American Diabetes Association (ADA) 2003 criteria¹⁶ as follows: (i) impaired fasting glucose (IFG), that is, fasting blood glucose (FBG) ≥ 100 and $< 126\text{mg/dL}$, (ii) impaired glucose tolerance test (IGT), that is, 2-hr glucose ≥ 140 and $< 200\text{mg/dL}$, and (iii) type 2 DM, that is, FBG $\geq 126\text{mg/dL}$ or 2-hr glucose $\geq 200\text{mg/dL}$. All subjects gave them informed consent to participate in the present study.

Statistical analysis

All analyses were performed with the SPSS statistical package version 10.0. Statistical significance

was set as 5%. All results are reported as mean \pm standard deviation. All parameters of three groups were compared each other with Mann-Whitney U Test. In statistically different parameters between groups, the difference producing group was determined by Bonferoni correction.

RESULTS

The mean age of groups were 21.27 ± 3.95 years for group 1, 24.46 ± 5.29 years for group 2, and 24.52 ± 6.90 years for group 3 respectively. There was not statically difference between groups for age ($p > 0.05$). The anthropometric characteristics of groups are listed in Table 1. Upon comparing both groups of patients; BMI, WHR were significantly increased in group 2 ($p = 0.07$). For metabolic profile, the mean of HOMA-IR was 3.09 ± 1.56 for group 2 and significantly low from other groups ($p = 0.01$). As OGTT determined mean value for group 2 was 127.32 ± 30.04 mg/dL and statically high from group 1 and 3 ($p = 0.05$). The value of OGTT was 102.18 ± 22.07 mg/dL for group 1 and 97.55 ± 25.28 for group 3. The metabolic characteristics of groups are listed in Table 2.

The outcomes of the Mann-Whitney U Test determined that there was statistically no difference between groups for FSH, E2, LH, TSH, FT3, FT4, DHEAS, As, Ftes, Ttes, progesterone on 21 th day of menstrual cycle. When we determined the interleukin-6 (IL-6) levels of the groups the mean for group 1 was 14.65 ± 6.18 pg/mL. The mean value for group 2 was 16.22 ± 6.55 pg/mL and 14.53 ± 7.32 for group 3. There was no statically difference between three groups for IL-6 ($p > 0.05$). The hormonal characteristics and IL-6 levels of groups are listed in Table 3. According to Bonferoni correction, the difference producing groups for BMI, WHR, FGS, HOMA-IR and for OGTT were determined as group 2 according to the others.

Table 1. The anthropometric characteristics of groups.

	Group 1 (n=22)	Group 2 (n=41)	Group 3 (n=22)	P value
Age (years)	21.27 ± 3.95	24.46 ± 5.29	24.52 ± 6.90	0.072
BMI (kg/m^2)	23.97 ± 2.73	26.55 ± 5.15	23.16 ± 3.62	0.017
WHR	0.76 ± 0.06	0.79 ± 0.07	0.75 ± 0.04	0.033
FGS	5.05 ± 3.80	9.00 ± 4.84	7.09 ± 4.07	0.005

BMI: Body mass index, WHR: Waist/Hip ratio, FGS: Ferriman-Gallwey score

Table 2. The metabolic characteristics of groups

	Group 1 (n=22)	Group 2 (n=41)	Group 3 (n=22)	P value
HOMA-IR	4,08±2,73	3,09±1,56	3,58±1,04	0.007
OGTT (mg/Dl)	102.18±22.07	127.32±30.04	97.55±25.28	0.001

HOMA-IR: insulin resistance by the homeostasis model assessment, OGTT: oral glucose tolerance test 120. minute value

Table 3. The hormonal characteristics and IL-6 levels of groups

	Group 1 (n=22)	Group 2 (n=41)	Group 3 (n=22)	P value
FSH (mIU/L)	5.23±1.77	5.88±2.66	6.28±4.01	0.664
E ₂ (pg/L)	94.93±79.66	88.11±121.64	107.54±120.84	0.902
LH (mIU/L)	10.80±9.83	11.95±9.38	9.94±5.94	0.318
TSH (mIU/L)	2.80±1.60	2.01±0.78	2.24±1.09	0.121
FT ₃ (pg/mL)	3.25±0.69	3.07±0.97	2.98±0.55	0.249
FT ₄ (pmol/L)	16.31±2.19	18.30±4.53	16.57±2.79	0.265
DHEA-S (ug/dL)	285.48±113.71	280.87±82.98	265.88±97.37	0.667
SHBG (nmol/L)	29.78±21.31	24.08±16.53	28.87±16.15	0.339
FTes (pg/mL)	3.30±3.07	3.41±2.30	3.18±2.79	0.411
TTes (ng/mL)	0.62±0.27	0.73±0.24	0.69±0.41	0.195
Prog-21 (ng/mL)	5.20±6.31	5.82±6.01	5.36±5.35	0.752
As (ng/mL)	4.35±1.60	4.05±1.51	3.57±1.12	0.205
IL-6 (pg/mL)	14.65±6.18	16.22±6.55	14.53±7.32	0.667

FSH: , follicle stimulating hormone, E₂: estradiol, LH: luteinizing hormone, FT₃: free triiodothyronine, FT₄: free thyroxine, DHEA-S: dihydroepiandrosterone sulphate, SHBG: sex hormone binding globuline, FTes: free testosterone, TTes: total testosterone, Prog-21: progesterone on 21th day of menstrual cycle, As: Androstenedion, IL-6: interleukin 6.

DISCUSSION

Polycystic ovary syndrome is one of the most common endocrine disorders in humans. In fact, most women with PCOS exhibit features of metabolic syndrome.¹⁷ The diagnosis of PCO is ultrasound examination of the ovaries with the presence of multiple peripheral small cysts. There is still debate about whether there has to be more than 10-12 cysts and whether both ovaries have to be effected. According to Rotterdam consensus criteria commonly used in clinical practice, two of the following three must be fulfilled for the diagnosis of PCOS: polycystic ovaries (12 or more follicles in each ovary, each follicle measuring 2-9 mm in diameter and/ or ovarian volume >10 mL, one polycystic ovary is sufficient for the diagnosis), oligo-/anovulation; clinically diagnosed as oligo-/amenorrhea and hyperandrogenism.¹

In this study, we analyzed the biochemical parameters and IL-6 according to the ovarian morphology in PCOS. Although there was not statistically a meaningful connection between the study groups, high levels at IL-6 were established in all groups. The process of an inflammatory response the invading pathogens or damaging insults is of critical importance to the homeostasis of the human body. Puder et al. demonstrated that the increase in low grade chronic inflammation and in insulin resistance in women with PCOS is associated with central fat excess. Independently of each other, both total body fat as well as central fat excess has a major impact on serum levels of inflammatory mediators, on the WBC, and on estimates of insulin resistance.¹⁸ There is a balance of IL-6 at inflammation, immunity, bone metabolism, natural development, reproduction and homeostasis. On the other hand, when the balance of IL-6 is damaged, the following pathologies are occurred as chronic inflammation,

autoimmunity, osteoporosis, atherosclerosis, rheumatoid arthritis, breast/prostate cancer, cardiovascular disease, diabetes and atopy.¹⁹ This inflammatory marker may be involved in the pathogenesis of the insulin resistance syndrome²⁰ and high levels of IL-6 become a risk for diabetes, atherosclerosis and cardiovascular disease.^{21,22} In the multi-ethnic study of atherosclerosis, psychosocial factors were positively associated with inflammatory markers including IL-6 concentrations, but these associations were reduced by 45-100 % after adjustment for BMI and diabetes.²³ As a result, it is believed that chronic inflammation is a predisposing factor for CVD. It has been correlated with obesity and insulin resistance but not hyperandrogenemia in PCOS.²⁴

In the presence of PCOS, there are many problems for the women. Hirsutism, acne, or alopecia is short-term problem, long-term effects are non-insulin-dependent diabetes mellitus, cardiovascular diseases (dyslipidemia, hypertension, vascular disease, ovarian dysfunction).²⁵ Ferriman Galway Score was determined significantly low at the group 1 of no ovarian pathology ($p=0.005$). Hirsutism increased with the level of ovarian pathology. The incidence of non-insulin-dependent diabetes mellitus is found six-fold at ²²⁻³¹ aged-women. Especially obesity is well recognized as a risk factor and is a common association with PCOS; diabetes is found a contributory cause for death 3, 6 times more commonly than expected. There were statistically significant connection between BMI and WHR (waist-hip ratio) at group 2 ($p=0.017$, 0.033 respectively). HOMA-IR was high level in all groups but that it was statistically found least in group 2 than the others was determined ($p=0.007$). The patients with or without ovarian morphology change the risk of type 2 diabetes does not change.

Many of the biochemical disturbances such as insulin resistance and hyperandrogenism and the resultant unfavorable changes in blood lipids, which are associated with PCOS, are recognized risk factors. PCOS may contribute to the risk of developing cardiovascular disease. Hyperinsulinemia has been found to be associated with a higher risk of cardiovascular disease.²⁶ The increased concentrations of inflammatory markers cluster with cardiovascular risk factors such as dyslipidemia, glucose intolerance and type 2 diabetes, hypertension, and obesity. Chronic low-grade inflammation has been proposed to play a role in the pathogenesis of insulin resistance and the metabolic syndrome.²⁷ Chronic inflammation could be involved in the development of metabolic syndrome and cardiovascular disease. Serum markers of chronic inflammation together

with insulin resistance are increasingly being considered as cardiovascular risk factors. Atherogenesis is associated with chronic local inflammation, and the inflammatory process could result in the release of cytokines into the circulation such as TNF- α or IL-6.²⁸

In conclusion, we could not find any difference between them in this clinical study, when we studied on clinic, hormonal and inflammatory markers according to the presence of the ovarian pathologies. PCOS had the same level of risk from CVS and diabetes mellitus for different ovarian morphology. IL-6 higher patients may much more risk of CVS and diabetes mellitus than the others. This situation might be said because of the findings both BMI and WHR for ovarian morphology difference at PCOS.

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