Polikistik Over Sendromlu hastalarda insülin direncinde Osteokalsin, TNF alfa ve adiponektinin rolü

AMAÇ: Polikistik Over Sendromu(PKOS)'nun temelinde yatan en önemli mekanizma insülin direncidir. Bu çalışmada PKOS'lu hastalarda kan osteokalsin düzeyinin;insülin direnci,adiponektin,TNF-alfa ve bazı hormonal parametrelerle olan ilişkisini değerlendirirmeyi amaçladık.

Gereç ve Yöntemler: PKOS tanımasına uyantı 44 hasta ve hiçbir rahatsızlığı olanımayan 16 sağlıklı kontrol grubundan oluşan toplam 60 hasta ile yapıldı. Bu hastaların kan osteokalsin, adiponektin,TNF-α, vücut kitle indeksi(VKİ), ve insulin direnci(IR) düzeyleri ölçülerek bu parametreler arasındaki ilişkiye incelemek için kullanıldı.

Bulgular: PKOS grubunda Homestasis model assessment (HOMA)-IR, adiponektin, osteokalsin ve androstenedion düzeyleri anlamlı olarak yüksek bulundu.VKİ ve HOMA-IR arasında pozitif ilişki bulunırken TNF-α ve osteokalsin, adiponektin ve VKİ arasında negatif ilişki saptandı.

Sonuç: PKOS'lu hastalarda kan osteokalsin düzeyinin adiponektin,TNF-α, ve IR üzerine etkisi bulunmaktadır. Saptanan farklı osteokalsin düzeyleri PKOS'lu hastalarda heterojeniteyi açıklamakta faydalı olabilir.

Anahtar Kelimeler: Polikistik over sendromu, insulin rezistansi, osteokalsin, adiponektin, tümör nekrozis faktör alfa

Interactions of Osteocalcin, Adiponectin, Tumor Necrosis Factor Alpha and Insulin Resistance in Polycystic Ovary Syndrome

Objective: Insulin resistance (IR) seems to be main pathogenic factor in polycystic ovary syndrome (PCOS). Adiponectin and tumor necrosis factor alpha (TNF-α) are important in IR. The aim of this study was to evaluate the correlations of osteocalcin, adiponectin, and TNF-α with IR in PCOS.

Materials and methods: A total of 60 women were divided into two groups. First group was constituted from 44 PCOS patients and control group was constituted from 16 healthy women. Osteocalcine, adiponectin, TNF-α levels, body mass index (BMI), and IR in the fasting state were assessed and correlations of these parameters were evaluated.

Results: Homestasis model assessment (HOMA)-IR, adiponectin, osteocalcin and androstenedione levels were significantly increased in PCOS group. Moderate positive correlation between BMI and HOMA-IR, moderate negative correlation between TNF-α and osteocalcin and mild negative correlation between adiponectin and BMI were detected in PCOS.

Conclusion: Osteocalcin may have impact on adiponectin, TNF-α and IR levels in PCOS. Different osteocalcin level in PCOS patients may be responsible for explaining PCOS heterogeneity.

Key words: Polycystic ovary syndrome, insulin resistance, osteocalcin, adiponectin, tumor necrosis factor alpha

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Manuscript Category: Reproductive Medicine and Endocrinology
Manuscript Type: Research
Precis: Bone is being recognized as an endocrine organ. Osteocalcin, seems to play a key role in the heterogeneity of PCOS.

ABSTRACT

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1. INTRODUCTION

PCOS is a common and heterogeneous disease characterized by anovulation, hyperandrogenism,
and/or polycystic ovaries (1,2). Therefore, an important consideration is whether such adipocytokines as adiponectin, a potential mediator of IR, are also implicated in the pathogenesis of PCOS (3). Levels of adiponectin, an abundant adipocyte-derived cytokine, are strongly correlated with measures of IR (4,5). Gonzalez et al. illustrated that hyperglycemia causes an increase in reactive oxygen species (ROS) generation from peripheral blood mononuclear cells (MNC) (6). ROS induced oxidative stress is a known activator of nuclear factor B (NFB), a proinflammatory transcription factor that promotes tumor necrosis factor (TNF) gene transcription (7). TNF is established as a mediator of IR by Hotamisligil et al. (8). Thus, increased TNF release from MNC in response to hyperglycemia may be an underlying mechanism for IR in PCOS.

Although previous animal studies showed that osteocalcin stimulated the expression of insulin in islets and of adiponectin in adipocytes with increased insulin secretion and sensitivity (9). Reduced osteocalcin levels have been claimed to be associated with diabetes mellitus (DM) development (10). We aimed to evaluate the correlations of blood osteocalcin, adiponectin, and TNF-α levels with IR in PCOS. Additionally, we evaluated the relationship of these with some of the hormonal parameters.

2. MATERIALS AND METHODS

A total of 60 women including 44 PCOS patients and 16 healthy women (control group) were studied at Erciyes University Gynecology Clinic. The diagnosis of PCOS was based on the established guidelines by consensus group for diagnosis of PCOS (1). Ultrasonographic diagnosis of polycystic ovaries was based on the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume >10 ml on pelvic or vaginal
ultrasound examination. Oligomenorrhea was defined as the absence of menstruation for 35 days or more and amenorrhea was defined as the absence of menstruation for 3 months or more (1).

All the women were examined both clinically and gynecologically including ultrasonography. Body weight, height, and BMI were recorded. The BMI was calculated as weight/(height)$^2$ in kilograms per square meter. Study and control groups were weight matched. Patients with congenital adrenal hyperplasia, androgen producing tumors, adrenal disfunction, Cushing's syndrome, hyperprolactinemia, DM, liver, kidney, heart, and thyroid diseases were excluded from the study. None of the women in study or control group had taken medications known to effect plasma sex steroids for $\geq$6 months before the study and none of the volunteers was cigarette smoker. All the women examined agreed to participate in the present study. This study was approved by the Ethics Committee of Erciyes University Hospital (2011-369) and a written informed consent form was obtained from each woman. Moreover we obtained Australian-New Zealand clinical trials registry number (ANZCTRN): 12613001132730. Venous blood from the subjects in a fasting state during the midfollicular phase of the menstrual cycle was collected between 8 and 9 am. Glucose levels three days after the normal diet and normal daily activity were measured by oxidase method with konelab 60-i auto-analyzers (Thermo Clinical L absystem Finland). IR in the fasting state was assessed by using HOMA and was calculated with the following formula: fasting plasma glucose (mmol/L) × fasting serum insulin (µU/mL) divided by 22.5, as described by Matthews et al. (11). Hormonal analyses included; thyroid stimulating hormone (TSH), dehydroepiandrosterone sulfate (DHEAS), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), 17-hydroxyprogesterone (17-OH-P), Androstenedione (A), free testosterone (fT), total testosterone (tT), insulin and sex hormone binding globulin (SHBG) levels. tT and fT (Biosource-Nivelles-Belgium), 17 OHP (DSL-3500, Texas/USA), DHEAS (Immunotech, Marseille/FRance), A (DSL-3800, Texas/USA) were measured by using an immunoradiometric assay method and its commercial kit, serum SHBG (Zentech, Angleur, Belgium), insülin (Biosource, Nivelles, Belgium), LH, FSH, P, PRL
(ACS:180, Bayer, Germany) were measured by using a chemiluminescence method and its commercial kit. After centrifugation, blood serum was stored at \(-70^\circ C\) until assayed. Adiponectin (Adiponectin kit, Assaypro, UK), TNF-\(\alpha\) (TNF-\(\alpha\) Invitrogen 96 Tests, UK) and osteocalcine (Gla-type osteocalcin invitro enzyme immunoassay kit, Takara Bio Inc., UK) were measured by using enzyme linked immunosorbent assay (ELISA) method.

The intra and inter-assay precision coefficients of variation were 2.8% and 4.6% for FSH, 5% and 6.2% for LH, 9.9% and 11.8% for E2, 4.4% and 4.8% for testosterone, 4.3% and 7.8% for fT, 11% and 2.8% and 7% for \(A_1\), 6.3% and 9.9% for DHEAS, 9.5% and 10.8% for 17-OHP, 5.2% and 5.8% for SHBG and 1.6% and 6.1% for insulin respectively. All results are expressed as means \(\pm\) SD.

2.1. Statistics

Shapiro-Wilk’s test was used to check the normality assumption of the data. Independent samples t test and Mann-Whitney U tests were used to compare the differences of variables between groups. Pearson and Spearman analysis were used to examine correlations and scatter-plot matrix was also given to display pairwise relationships between variables. To identify the independent risk factors of PCOS, univariate and multivariate logistic regression analysis was used and odds ratios (ORs) were calculated with their 95% confidential intervals (CIs).

Statistically significant variables in univariate analysis were included in the multivariate logistic model and backward stepwise selection was performed at a stringency level of \(p<0.10\) to determine the independent risk factors on PCOS. Two-sided \(p\) values \(<0.05\) were considered statistically significant.

3. RESULTS

Study group and control group were weight matched. Hormone levels and baseline characteristics of groups were illustrated in Table 1.
Level of A was significantly high in PCOS group. There were no statistically significant difference between groups for age, BMI, DHEAS, FSH, SHBG, LH, fT, tT and E2. High levels of HOMA-IR, adiponectin and osteocalcin were detected in PCOS group. There was no significant difference between two groups for TNF-α (Table 1). The cut of the value of HOMA-IR was accepted as 2.5 (12,13).

We detected a strong positive correlation between adiponectin and osteocalcin in control group. There was positive correlation between osteocalcin and BMI in addition to negative correlation between osteocalcin and TNF-α in PCOS group. We found moderate positive correlation between BMI and HOMA-IR, moderate negative correlation between TNF-α and osteocalcin and mild negative correlation between adiponectin and BMI (Table 2, Figure 1).

4. DISCUSSION

Many of the symptoms appear to be quite heterogeneous; with marked differences in their prevalence and intensity among different groups of women with PCOS. IR was significantly high in PCOS group. Some studies show IR only in obese PCOS women (14) and others demonstrate IR in lean PCOS patients (15). Of importance, the studies which failed to demonstrate IR in lean PCOS women did, however, demonstrate elevated basal insulin levels compared to weight matched, non PCOS controls (14).

Groups were weight matched in the study therefore the effect of adipose tissue on TNF-α and adiponectin were eliminated. We found higher levels of adiponectin in PCOS; however, some of the authors suggest that women with PCOS had lower adiponectin level (15). Conversely, an increment in plasma adiponectin was obtained by Frystyk et al. in type 1 DM (16). One way to interpret the present findings is to conclude that high adiponectin levels may be an early predictor of DM development. Unfortunately, %52.7 of patients in PCOS group had IR.
However, the finding can also be interpreted in the opposite way, as elevated adiponectin levels could represent a beneficial compensatory mechanism. Several markers of inflammation are increased in PCOS, suggesting that it is a state of chronic low grade inflammation. Having the anti-inflammatory and anti-DM properties of adiponectin in mind, one could hypothesize that increased adiponectin levels serve to protect patients at high risk of the harmful actions of pro-inflammatory and DM agents.

Increased levels of TNF-α were detected in PCOS however there was no statistical difference between groups. Although Vural et al. couldn’t illustrate higher TNF-α level in PCOS (17), Xiong et al. suggested that patients with PCOS showed significantly higher serum TNF-α level (18). The pathogenic impact of TNF-α in IR state is underscored by the effect of the functional polymorphisms in the promoter regions of TNF-α, with different transcription rates (19), or this situation may be related to balance of anti-inflammatory and inflammatory agents that are secreted by bone, adipose tissue etc. in PCOS.

More recently, evidence from animal studies suggests that the skeleton may exert an endocrine regulation of glucose metabolism (20). Lee et al. showed that mice lacking the gene that encodes osteocalcin have an abnormal amount of visceral fat and exhibit glucose intolerance, IR, and impaired insulin secretion compared with wild-type mice (20). Adami et al. couldn’t illustrate significant difference between PCOS and control group for osteocalcin but they found normal androgen levels in their PCOS group; additionally, they didn’t examine patients for IR (21). In our study osteocalcin was significantly increased in PCOS; moreover, there were negative correlation between osteocalcin and TNF-α. Our study is in agreement with Diamanti-Kandarakis et al., who illustrated higher osteocalcin levels in PCOS (22).

There were no correlation between serum adiponectin and HOMA-IR. There were moderate negative correlation between osteocalcin and TNF-α in addition to moderate positive correlation between BMI and HOMA-IR in PCOS. Adiponectin secretion is strongly related with IR rather than obesity and previous animal study showed that osteocalcin stimulated the expression of...
insulin in islets and of adiponectin in adipocytes with increased insulin secretion (23). Perhaps increased osteocalcin levels contribute to high HOMA-IR by increased insulin secretion. Our groups were weight matched and source of adiponectin is adipose tissue, this situation may explain why we didn’t detect a correlation between serum adiponectin levels and HOMA-IR.

TNF-α can be released from MNC and hyperglycemia causes an increase in ROS generation from MNC. Osteocalcin is defined in literature with antidiabetic and anti-inflammatory properties, so plausible explanation of these events are increased osteocalcin levels lead to decrease TNF-α. Osteocalcin level may have impact on adiponectin, TNF-α and IR in PCOS. Therefore, osteocalcin may be responsible for explaining PCOS heterogeneity.

Our results, due to relatively small sample size, display only weight matched controls in PCOS. There is a need for further, larger scale studies including interacting with other genetic and environmental factors and development of PCOS.

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Declaration of interest: There is no conflict of interest among authors.

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5. REFERENCES


Table 1: Hormonal levels and baseline characteristics of groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Between group comparisons</th>
<th>Logistic regression analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCOS group (n=44)</td>
<td>Control group (n=16)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.50(20.00-24.00)</td>
<td>22.50(19.50-24.75)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.50(20.25-26.00)</td>
<td>21.00(20.00-23.00)</td>
</tr>
<tr>
<td>tT (pg/ml)</td>
<td>80.50(49.75-113.25)</td>
<td>72.00(61.25-93.00)</td>
</tr>
<tr>
<td>fT (pg/ml)</td>
<td>2.64±0.83</td>
<td>2.22±1.24</td>
</tr>
<tr>
<td>A (ng/ml)</td>
<td>3.05(2.27-4.12)</td>
<td>2.24(2.00-2.98)</td>
</tr>
<tr>
<td>DHEAS (ng/ml)</td>
<td>2182.34±125.44</td>
<td>2334.37±768.27</td>
</tr>
<tr>
<td>SHBG (nmol/ml)</td>
<td>47.00(26.50-95.50)</td>
<td>49.50(41.25-85.75)</td>
</tr>
<tr>
<td>FSH (pg/ml)</td>
<td>5.00(4.00-4.00)</td>
<td>5.30(4.45-7.75)</td>
</tr>
<tr>
<td>LH (pg/ml)</td>
<td>5.30(4.42-9.00)</td>
<td>8.00(5.30-11.75)</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>77.00(50.00-95.00)</td>
<td>73.00(57.00-105.00)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.35(1.50-3.20)</td>
<td>1.35(1.08-2.37)</td>
</tr>
<tr>
<td>Variable</td>
<td>Control Group</td>
<td>PCOS Group</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>64.67(61.08-68.18)</td>
<td>60.52(59.16-62.30)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>33.02(12.29-86.05)</td>
<td>15.46(13.13-29.08)</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>1.96(1.45-2.36)</td>
<td>1.01(0.79-1.39)</td>
</tr>
</tbody>
</table>


Table 2: Correlation of osteocalcin level with hormonal levels, age, BMI, HOMA-IR, TNF-α and adiponectin for both groups.
<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>BMI: 0.068(0.802)</th>
<th>BMI: -0.333(0.027)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.040(0.882)</td>
<td>0.638(0.027)</td>
<td></td>
</tr>
<tr>
<td>tT</td>
<td>0.066(0.80)</td>
<td>0.175(0.257)</td>
<td></td>
</tr>
<tr>
<td>fT</td>
<td>-0.001(0.996)</td>
<td>0.055(0.725)</td>
<td>305 tT: Total testoster</td>
</tr>
<tr>
<td>A</td>
<td>0.234(0.383)</td>
<td>-0.139(0.367)</td>
<td>Free testoster one, fT:</td>
</tr>
<tr>
<td>SHBG</td>
<td>-0.341(0.196)</td>
<td>-0.041(0.793)</td>
<td></td>
</tr>
<tr>
<td>DHEAS</td>
<td>0.200(0.458)</td>
<td>0.022(0.889)</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>-0.086(0.752)</td>
<td>-0.054(0.726)</td>
<td>310 FSH: Follicle-stimulating hormone,</td>
</tr>
<tr>
<td>LH</td>
<td>0.021(0.940)</td>
<td>0.154(0.318)</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>0.337(0.201)</td>
<td>0.054(0.727)</td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.671(0.004)</td>
<td>-0.061(0.695)</td>
<td></td>
</tr>
<tr>
<td>TNF- α</td>
<td>0.344(0.192)</td>
<td>-0.338(0.025)</td>
<td></td>
</tr>
<tr>
<td>HOMA- IR</td>
<td>0.091(0.736)</td>
<td>-0.155(0.314)</td>
<td>315 HOMA-IR: Homestasis model assessment insulin resistance,</td>
</tr>
</tbody>
</table>

Figure 1: A scatter plot matrix to display the relationship among BMI, HOMA-IR, TNF-α, adiponectin and osteocalcin variables (*p< 0.05, **p< 0.01)