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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

POLİKİSTİK OVER SENDROMLU HASTALARDA SERUM SKLEROSTİN VE DİCKKOPF-1 SEVİYELERİ

SERUM SCLEROSTIN AND DICKKOPF-1 LEVELS IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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ÖZET

AMAÇ: Polikistik over sendromu (PKOS), premenopozal kadınlarda en sık görülen endokrin patolojidir ve çeşitli sistemleri etkileyen karmaşık bir sendromdur. PKOS'un kemik metabolizması üzerindeki etkileri hakkında çok sayıda çalışma yapılmıştır, ancak PKOS'da osteoporoz riski konusundaki veriler çelişkilidir. Wingless-type mouse mammary tumor virus integration site (Wnt) yolu, kemik metabolizmasının düzenlenmesinde önemli rol oynar. Bu yolun inhibitörleri olarak Sklerostin (Scl) ve Dickkopf-1 (DKK1) son zamanlarda osteoporozun terapötik tedavisi için hedefler haline gelmiştir. Bu çalışma, PKOS'lu kadınlarda Scl ve DKK1 düzeylerini belirlemeyi amaçlamıştır.

GEREÇ VE YÖNTEM: Bu çalışmada PKOS tanısı konulmuş 36 kadın ve 35 sağlıklı gönüllü retrospektif olarak incelendi. Her iki grup, demografik, antropometrik, biyokimyasal parametrelerin yanı sıra Scl ve DKK1 seviyeleri açısından karşılaştırıldı.

BULGULAR: Scl seviyesi, PKOS grubunda 42,68 ± 13,28 pg / mL ve kontrol grubunda 45,69 ± 11,79 pg / mL idi ve istatistiksel olarak benzerdi. DKK1 seviyesi, PKOS grubunda 1444,73 ± 611,30 pg / mL ve kontrol grubunda 1204,26 ± 660,88 pg / mL idi ve istatistiksel olarak benzerdi. PKOS grubunun vücut kitle indeksi (VKİ) ve bel/kalça oranı (BKO) değerleri, istatistiksel olarak anlamlı olmamasına rağmen, kontrol grubuna göre daha yüksekti.

SONUÇ: Bu çalışma, PKOS'lu kadınlarla sağlıklı bireyler arasında, Scl ve DKK1 düzeyleri bakımından anlamlı bir fark olmadığını göstermiştir. Amenore PKOS olgularında kemik kaybına neden olsa da, hiperandrojenemi ve hiperöstrojeneminin kemik yoğunluğu üzerindeki olumlu etkileri dengeleyici bir unsur olarak kabul edilebilir.

ANAHTAR KELİMELER: Polikistik over sendromu, Osteoporoz, Sclerostin, Dickkopf-1.

ABSTRACT

OBJECTIVE: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in premenopausal women, and it is a complex syndrome affecting various systems. Numerous studies have been carried out, on the effects of PCOS on bone metabolism, but currently, there is no clear information about the risk of osteoporosis in PCOS. The wingless-type mouse mammary tumor virus integration site (Wnt) pathway plays an important role in the regulation of bone metabolism. As inhibitors of this pathway, Sclerostin (Scl) and Dickkopf-1 (DKK1) have recently become therapeutic targets for osteoporosis treatment. This study aims to determine Scl and DKK1 levels in women with PCOS.

MATERIAL AND METHODS: In this study, 36 women diagnosed with PCOS and 35 healthy volunteers were examined retrospectively. Both groups were compared in terms of demographic, anthropometric, and biochemical parameters as well as Scl and DKK1 levels.

RESULTS: The level of Scl was 42,68 ± 13,28 pg/mL in the PCOS group and 45,69 ± 11,79 pg/mL in the control group, indicating no statistically significant difference. The level of DKK1 was 1444,73 ± 611,30 pg/mL in the PCOS group, and 1204,26 ± 660,88 pg/mL in the control group, indicating no statistically significant difference. The body mass index (BMI) and waist to hip ratio (WHR) values of the PCOS group were higher than the control group, although these differences did not reach statistical significance.

CONCLUSIONS: This study shows that there were no significant differences between women with PCOS and healthy individuals in terms of Scl and DKK1 levels. Although amenorrhea causes bone loss in PCOS patients, the positive effects of hyperandrogenemia and hyperestrogenemia on bone density can be regarded as a balancing effect.

KEYWORDS: Polycystic ovary syndrome, Osteoporosis, Sclerostin, Dickkopf-1.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most frequently observed endocrine disorder in women (1, 2). It is a complex syndrome characterized by biochemical or clinical hyperandrogenism, oligo-anovulation, and insulin resistance. It leads to problems such as menstrual irregularities, hirsutism, infertility, obesity, dyslipidemia, and can cause type II diabetes mellitus (DM) and cardiovascular disorders in later life (3, 4). How bone metabolism is affected by PCOS is not yet clear. Many hormonal changes and body-composition changes that can affect bone metabolism occur in PCOS. There are several studies showing that bone mineral density (BMD) increases (5, 6), remains unchanged (7-9), or decreases (10, 11) in PCOS patients. It can be speculated that hyperandrogenism, obesity and hyperinsulinemia prevent osteoporosis in PCOS patients; however, amenorrhea, increased cortisol, hypovitaminosis D and a decrease in growth hormone levels lead to decreased BMD (10, 12).

The Wnt pathway has a central role in the determination of bone mass. Pathways triggered upon wingless-type mouse mammary tumor virus integration site (Wnt) activation lead to an increase in osteoblastic activity (13). The Wnt inhibitors, such as Sclerostin (Scl) and Dickkopf-1 (DKK1) bind low-density lipoprotein receptor-related protein (LRP) 5/6 co-receptors, inhibiting their interaction with Wnt. It has been also shown that mutations which cause overexpression of Scl and DKK1 lead to the development of osteoporosis (14). Inhibition of Scl and DKK1 with specific antibodies and inhibitors has also a protective effect against osteoporosis, increasing bone production (15).

There are no studies in the literature on how PCOS, which affects numerous metabolic pathways and has controversial effects on the bone, affects the Wnt pathway, which plays a critical role in bone metabolism. The present study aims to identify how Scl and DKK1 levels change in women with PCOS.

MATERIALS AND METHODS

A total of 36 treatment-naive women aged 18 to 49 years who were newly diagnosed with PCOS and were admitted to endocrinology and metabolic diseases outpatient clinics were assigned as the patient group. The control group consisted of 35 healthy women without any complaints or diseases, who were admitted to the internal medicine outpatient clinic for a routine check-up. In order to avoid the possibility that BMI could affect the study results, healthy subjects with BMI similar to the study group were included in the study as the control group. Written informed consent was obtained from each participant and the study was conducted in accordance with the principles of the Declaration of Helsinki.

The diagnosis of PCOS was made using the 2003 Rotterdam criteria (16). These criteria are defined as: the presence of oligo-anovulation, clinic and/or biochemical hyperandrogenism and ultrasonography (USG) detection of polycystic ovaries, once all the other causes which can lead to a similar clinical presentation are ruled out. Polycystic ovary syndrome is defined as the presence of two of three criteria. Exclusion criteria were as follows: thyroid disease, hyperprolactinemia, Cushing's disease, drug-related PCOS, and non-classical congenital adrenal hyperplasia. Having DM, pregnancy, kidney and liver dysfunction, accompanying cardiovascular disease, malignancy, inflammatory diseases, or other metabolic diseases; currently using oral contraceptives or having used them in the last 6 months, using glucocorticoids, anti-androgens, drugs for the induction of ovulation, anti-diabetics, obesity drugs, and other hormonal supplements were also considered as exclusion criteria. The control group included participants with no menstrual irregularities, no hirsutism and any other known diseases, no use of oral contraceptives or other drugs, no history of hormonal treatment, and who gave their last birth three or more years ago. Because obesity and thinness can affect bone density, the control group was selected from those with a BMI similar to the PCOS group. Those who had any pathological findings other than excess body weight on physical examination were excluded.

Hormonal tests were not performed on the healthy control group. Menstrual cycles, BMI, WHR, history of acne, infertility, and male pat-

tern hair loss were assessed in both groups. Oligomenorrhea was defined as a menstrual cycle longer than 35 days with less than nine menstrual cycles in a year. Ferriman Gallwey Scoring (FGS) system was used to score the degree of hirsutism by an endocrinologist (17). The following baseline hormone levels were measured in women with PCOS: luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), total testosterone (tT) and Dehidroepiandrosteron Sülfat (DHEA-S). DHEA-S < 332 µg/dL (female) and tT < 0.6 ng/mL (female) were evaluated as normal (no hyperandrogenism). Sonographic appearance of ovaries with 12 or more 2 to 9 mm follicles or with a volume of more than 10 mL was accepted as polycystic.

In order to measure Scl and DKK1 levels, venous blood samples of all patients and controls were collected following 12 hours of fasting between 08.00-09.00 A.M. Of all patients with PCOS, those with a regular menstrual cycle were tested in the early follicular phase, while those with amenorrhea were randomly tested for fasting glucose, FSH, LH, E2, tT, PRL, DHEA-S, 17-hydroxyprogesterone, and thyroid-stimulating hormone (TSH) levels. Serum Scl levels were specified by commercial kits (ab155440,Abcam, Cambridge, UK), and DKK1 levels were measured by commercial kits (ab100501, Abcam, Cambridge, UK) based on enzyme-linked immunosorbent assay (ELISA). The results were read using an ELISA reader (model ELX 808 IU(-BioTek® Instruments, Winooski, VT, USA). Intra- and inter-day coefficients of variation for Scl and DKK1 were <%10 and <%12, respectively.

Ethical Committee

The study protocol was approved by the Ethics Committee of Canakkale Onsekiz Mart University, Clinical Research Ethics Committee (No. 05099-242/ 25.12.2014).

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 19.0 software IBM Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation. The Kolmogorov-Smirnov test was used to analyze normally distributed variables between the groups. Student t-test, Mann-Whitney U test, and Spearman's correlation test were used for the analyses. A p value of <0.05 was considered statistically significant.

RESULTS

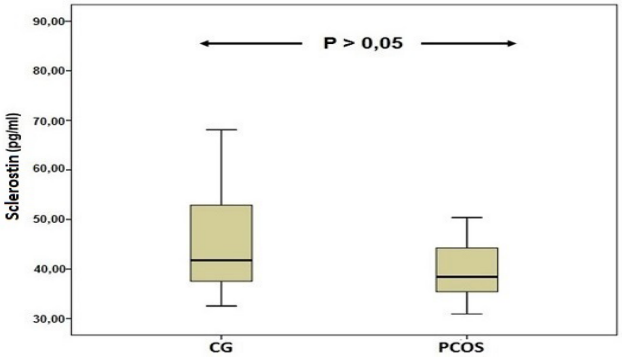
Table 1 displays that there were no statistically significant differences in the mean age and BMI values of PCOS and control groups (p>0,05). However, the FGS score was significantly higher in the PCOS group than in the control group (p<0.001) **(Table 1)**.

Table 1: Clinical Characteristics of PCOS Patients and Healthy Controls. (Scl, DKK, demographic, anthropometric and biochemical measurements)

PCOS	Control (n:36) Mean(SD)	P (n:35) Mean(SD)	
Age (year)	24,02 ± 5,52	25,77 ± 6,26	P>0,05**
BMI (kg/m2)	26,61 ± 5,92	23,25 ± 3,20	P>0,05 **
WHR	0,80 ± 0,06	0,77 ± 0,06	P>0,05 **
Glucose (mg/dL)	88,61 ± 5,24	(*)	
FSH (mIU/mL)	6,59 ± 3,18	(*)	
LH (mIU/mL)	8,27 ± 4,55	(*)	
PRL (ng/mL)	19,59 ± 10,12	(*)	
DHEAS (µg/dL)	279,97 ± 130,91	(*)	
tT (ng/mL)	0,44 ± 0,18	(*)	
TSH (mIU/mL)	2,37 ± 0,97	(*)	
E2 (pg/mL)	1,15 ± 29,52	(*)	
17-OH P (ng/mL)	0,95 ± 0,48	(*)	
FGS	13,25 ± 6,03	1,1 ± 1,3	P<0.001**
Scl (pg/mL)	42,68 ± 13,28	45,69 ± 11,79	P>0,05***
DKK-1 (pg/mL)	1444,73 ± 611,30	1204,26 ± 660,88	P>0,05***

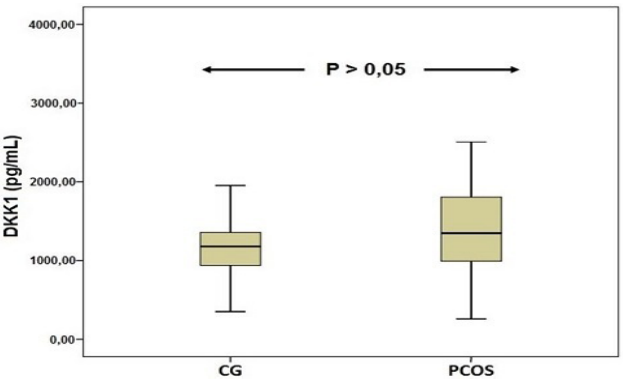
BMI: Body mass index, WHR: Waist–hip ratio, FSH: Follicle-stimulating hormone, LH: Luteinizan hormone, PRL: Prolactin, DHEAS: Dehydroepiandrosterone sulfate, tT: Total Testosterone, TSH: Thyroid-stimulating hormone, E2: Estradiol, 17-OH P:17-hydroxy progesterone, FGS: Ferriman-Gallwey Scoring, Scl: sclerostin, DKK-1: Dickkopf-1, (*): Not measured , **independent Student t-test *** Mann-Whitney U test, P<0.05 significant

Serum Scl level was 42,68 ± 13,28 pg/mL in PCOS group and 45.69 ± 11.79 pg/mL in control group, indicating no statistically significant difference **(Figure 1)**.



Figüre 1: Sclerostin levels of PCOS and control groups. CG: Control group, PCOS: Polycystic over syndrome group

Serum DKK1 level was 1444,73 ± 611,30 pg/mL in PCOS group, and 1204,26 ± 660,88 pg/mL in control group, indicating no statistically significant difference **(Figure 2)**.



Figüre 2: Dickkopf-1 levels of PCOS and control groups. CG: Control group, PCOS: Polycystic over syndrome group

As for the subgroup analysis, no significant differences in Scl and DKK1 levels were observed between obese PCOS patients (BMI ≥30 kg/m^2) and non-obese PCOS patients (BMI <30 kg/m^2) (p=0,20 and 0,60, respectively). Moreover, there were no significant differences in Scl and DKK1 levels of PCOS patients with and without hirsutism (p=0,49 and 0,14, respectively).

Correlation analyses showed that DHEA-S levels significantly correlated with Scl and DKK1 levels (p= 0,027 and 0,002, respectively). While there was no correlation between E2 and Scl (p=0.025), no correlation was observed between E2 and DKK (p=0.165).

DISCUSSION

This study investigates how the levels of Scl and DKK1, natural inhibitor proteins of Wnt signaling, are altered in PCOS patients. To the best of our knowledge, this is the first study to examine how the Wnt signaling pathway, which has a recently understood role in bone metabolism, is affected by PCOS (13).

Sclerostin acts locally, but circulating levels may reflect changes in bone (18). One study found a significant positive correlation between serum sclerostin levels and bone mineral content (BMC), bone mineral density, BMI, and android/gynoid fat. (19). This study has been unable to find any significant difference in Scl and DKK1 levels of PCOS patients and healthy controls.

This finding is consistent with the previous findings showing that BMD remained unchanged in PCOS patients (Adami et al.1998, Good et al.) (8, 9). In the study by Adami et al. (8), BMD values of PCOS patients were similar to the control group, although BMD values of amenorrheic PCOS patients were lower. The aforementioned study also showed that BMD correlated with DHEA-S and tT levels. The authors concluded that hyperandrogenism was protective against bone loss due to oligo/amenorrhea in PCOS patients. However, in our study, DHEA-S and tT levels were within the normal range in PCOS patients without biochemical hyperandrogenism.

The fact that androgen levels were not high in our study may be due to the fact that the Rotterdam criteria, in which we selected PCOS patients, do not require hyperandrogenism, and the patients who come to our outpatient clinic are usually patients with menstrual irregularity. In addition, we found that DHEA-S correlated with Scl and DKK1 levels in PCOS patients but not with tT levels. Considering the fact that Scl and DKK1 levels increase in a positively-correlated manner with DHEA-S, this finding suggests that increased DHEA-S levels reduce the activation of the Wnt signaling pathway, thereby, inhibiting bone production. However, this finding was contradictory to the results of Adami et al. (8) indicating that high androgen levels had a positive correlation with high BMD values.

In another study, Di Carlo et al. (5) showed that BMD values in PCOS patients were higher than in those with PCOS-unrelated amenorrhea and healthy volunteers. In this study, BMI values in the PCOS group were significantly higher than in all the other groups. However, in our study, BMI values in the PCOS group were statistically similar to the control group. Moreover, subgroup analysis failed to show any statistically significant difference in Scl and DKK1 levels of PCOS patients with respect to obesity. On the other hand, this result contradicted the findings of Di Carlo et al. (5) suggesting that Scl and DKK1 levels were expected to reduce if obesity was a factor which increased bone production.

In another study by Dagogo et al. (6), a total of 32 women with hirsutism and ten women with PCOS were evaluated. In that study, BMD values were significantly higher in women with hirsutism, compared to those without hirsutism. However, no correlation was detected between hirsutism and Scl and DKK1 levels in our study. What is more, subgroup analysis failed to show any statistically significant difference in Scl and DKK1 levels of PCOS patients in the aspect of hirsutism, but this finding did not comply with the findings of Dagogo et al. (6).

This study is firstly limited by the absence of longitudinal data. The second major limitation is the inability to perform bone mineral densitometry. Due to the ethical concerns and lack of indication, none of the participants in this study could undergo bone mineral densitometry. Therefore, we have been unable to compare serum Scl and DKK1 levels on the basis of BMD values.

In conclusion, this study shows that there were no significant differences in Scl and DKK1 levels between PCOS patients and healthy women. Although amenorrhea causes bone loss in PCOS patients, the positive effects of hyperandrogenemia and hyperestrogenemia on bone density appear to be balance out this harmful impact. However, large-scale studies are needed to clarify the role of the Wnt pathway in various phenotypes of PCOS.

Ogün İrem Bilen, Yıldız Garip Bilen, Mustafa Eroğlu, Hakan Türkon, Yasemin Sefika Akdeniz and Mehmet Asik declare that they have no conflict of interest and no sponsor.

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