Türk Kadın Sağlığı ve Neonatoloji Dergisi

To cite this article: Arda Duz S, Tuncay G, Karaer A. The determination of anti-mullerian hormone and vitamin D serum levels in polycystic ovary syndrome. Turk J Womens Health Neonatol 2020; 2(1): 1-6

Original Article

The determination of anti-mullerian hormone and vitamin D serum levels in polycystic ovary syndrome†

Polikistik over sendromunda anti-müllerian hormon ve vitamin D düzeylerinin değerlendirilmesi

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Abstract

Aim: This study aimed to determine whether there is a relationship between serum anti-mullerian hormone (AMH) and vitamin D levels and the severity of polycystic ovary syndrome (PCOS).

Material and method: Forty-four women with PCOS and forty-four controls with regular ovulatory menstrual cycles were included in this study between February 2016 and November 2016. Hormonal parameters, glucose metabolism parameters, clinical signs, and symptoms and serum AMH and vitamin D levels were determined.

Results: AMH levels, hirsutism scores, and postprandial glucose levels were significantly different between the two groups. There were statistically significant positive correlations between AMH and luteinizing hormone (LH)/follicle stimulating hormone (FSH) ratio, homeostatic model assessment of insulin resistance (HOMA-IR), fasting glucose levels, and hirsutism scores. Serum AMH levels were significantly higher in women with PCOS compared to controls. The levels of vitamin D were found low in both groups, and there was no statistically significant difference between the two groups in vitamin D levels.

Conclusion: There were positive correlations between AMH levels and LH/FSH ratio, hirsutism scores, HOMA-IR, and the fasting insulin. The patients with higher AMH levels were more hyperandrogenic compared to patients with PCOS who have lower AMH levels. Nevertheless, there was no statistically significant difference between the two groups in vitamin D levels.

Key words: Anti-mullerian hormone; hirsutism; hyperandrogenism; polycystic ovary syndrome; vitamin D

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†The abstract of this study was presented as an oral presentation at III. International Expermed Congress, on 11-14 April 2019, in Bafra, Cyprus.

Öz

Amaç: Bu çalışmanın amacı polikistik over sendromu (PKOS) ile serum anti-müllerian hormon (AMH) ve D vitamini düzeyleri arasında klinik açısından bir ilişki olup olmadığının araştırılmasıdır.

Gereç ve yöntem: Şubat 2016 ile Kasım 2016 tarihleri arasında 44 PKOS tanısı almış hasta ile normal menstrüel siklusları olan 44 kontrol grubu hastası çalışmaya dahil edilmiştir. Hormonal parametreler, glukoz metabolizması parametreleri, klinik bulgu ve belirtiler, serum D vitamini ve AMH düzeyleri değerlendirilmiştir.

Bulgular: AMH düzeyleri, hirsutism skorları ve tokluk kan şekerleri iki grup arasında istatistiksel olarak anlamlı bir şekilde farklıydı. AMH ile luteinize edici hormon (LH)/folikül stimule edici hormon (FSH) oranı, insulin direnci homeostatik model değerlendirmesi (HOMA-IR), açlık kan şekeri ve hirsutizm skorları arasında pozitif korelasyon tespit edildi. PKOS grubunda serum AMH düzeyleri kontrol grubuna göre anlamlı olarak daha yüksek saptandı. Serum D vitamini seviyeleri her iki grupta düşük saptanmakla birlikte gruplar arasında D vitamin düzeyleri açısından fark yoktu.

Sonuç: Serum AMH seviyeleri ile LH/FSH oranı, hirsutism skoru, HOMA-IR ve açlık insulin düzeyleri arasında pozitif korelasyon bulunmuştur. Daha yüksek AMH seviyelerine sahip olan hastaların, PKOS olup da düşük AMH seviyesine sahip olanlara göre daha hiperandrojenik olduğu bulunmuştur. Bunların yanı sıra gruplar arasında, vitamin D düzeyleri açısından istatistiksel olarak anlamlı fark saptanmamıştır.

Anahtar kelimeler: Anti-müllerian hormon; hirsutizm; hiperandrojenizm; polikistik over sendromu; vitamin D

Introduction

Polycystic ovary syndrome is the most common endocrine disorder in women during the reproductive ages and is often accompanied by insulin resistance and hyperinsulinemia (1).

Recently there has been a focus on vitamin D supplementation as an adjuvant treatment of PCOS. Indeed, women with PCOS have been found to have a high prevalence of vitamin D deficiency. Additionally, some studies have found a correlation between serum vitamin D levels and several metabolic symptoms in women with PCOS, such as type 2 diabetes mellitus (2-5).

Vitamin D has been thought to have a role in fertility through its action on ovarian function and on the immune system. The vitamin D receptor (VDR) has been identified in reproductive cells, such as endometrial and ovarian granulosa cells (6).

To date, several clinical trials have evaluated the effects of vitamin D on women with PCOS. There is some, but limited, evidence for beneficial effects of vitamin D supplementation on insulin resistance, ovarian follicles maturation, ovulation and menstrual regularity in women with PCOS (7,8).

Women with PCOS have high concentrations of anti-mullerian hormone (AMH) (9). AMH is a glycoprotein produced in the granulosa cells of the ovary that regulates early follicular recruitment (10). Preantral and small antral follicles secrete AMH, and there is a good correlation between AMH and ovarian follicle count (11). Recent studies focus on the determination of the relationship between AMH and PCOS as well as the clinical utility of serum AMH as an adjunct test in the diagnosis of PCOS (12-14). In a recent meta-analysis, symptomatic PCOS patients have serum AMH levels higher than 4.7 ng/ml (9).

This study aims to determine serum vitamin D and AMH levels in PCOS patients and to investigate the correlation between serum levels of these two factors and the severity of the syndrome.

Material and Method

Between February 2016 and November 2016, 44 patients, diagnosed with PCOS in our outpatient gynecology clinic, were recruited in this study. The number of patients was calculated by a power analysis. The local ethics committee approved the study (Protocol code: 2016/187).

The inclusion criteria were: (a) the women between 18-40 years old, (b) absence of medication use in the last three months that would affect hormonal and/or insulin metabolism, (c) absence of systemic and/or metabolic disease. The exclusion criteria were: (a) thyroid dysfunction, hyperprolactinemia, congenital adrenal hyperplasia or adrenal tumors, (b) a chronic systemic disorder such as type 1 or 2 DM or hypertension, (c) body mass index >35 kg/m^2.

The diagnosis of PCOS was considered based on the presence of at least two criteria of Rotterdam: (a) oligo- and/or anovulation, (b) the presence of the clinical and/or biochemical markers of hyperandrogenism, (c) polycystic ovaries feature in ultrasonography (USG) (15).

Oligomenorrhea was defined as menstrual cycles longer than 35 days, and amenorrhea was defined as no menstrual bleeding for three consecutive periods. Hirsutism is a clinical symptom of hyperandrogenism, and in this study, it was evaluated using the Ferriman-Gallwey method, and a score higher than eight was defined

as hirsutism (16). The presence of at least 12 peripheric placed follicles having a size between 2 and 9 mm was defined as polycystic ovaries.

Age, height, and weight of the patients were recorded during the physical examination. Body mass index (BMI) was calculated as: BMI = weight [kilograms] / square of height [meters].

On the 3rd day of the menstrual cycle, at 8:00-9:00 am, after a three day of normal carbohydrate diet and a minimum of 10 hours overnight fasting, plasma insulin and glucose levels, hormonal profile, vitamin D and AMH levels were evaluated. IR (Insulin Resistance) was determined with Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) as (17): HOMA-IR = fasting glucose (mg/dL) x fasting insulin (pmol/l)/405. Serum fasting glucose and postprandial second-hour glucose levels were determined by the spectrophotometric method (Aeroset, Abbott Laboratories, Abbott Park, IL). Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin, thyroid-stimulating hormone (TSH), total testosterone, free testosterone, 17-OH-progesterone, dehydroepiandrosterone sulfate, vitamin D, AMH and fasting insulin levels were determined by chemiluminescence method (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA).

Vitamin D levels that under 20 ng/mL were assessed as deficient, between 20 and 30 ng/mL evaluated as insufficient, and more than 30 ng/mL estimated as sufficient (18).

The data were analyzed using the Statistical Package for Social Sciences software 17.0 (SPSS, Inc., Chicago, IL). The normality of the distribution of variables was tested using the Kolmogorov-Smirnov test. Student-T test was used for the variables which had a normal distribution, and the Mann-Whitney-U test was used for the variables which did not have a normal distribution. The Chi-square test was used to analyze the categorical data. All data were referred to as the median (interquartile range) and mean ± standard deviation (SD). A p-value < 0.05 was considered as statistically significant.

Results

The women with PCOS were named Group 1, and control patients were called Group 2. **Table 1** indicates the median age and BMI of the patients in Group 1 and 2. There was no statistically significant difference in terms of age and BMI between the groups.

Table 1: The median age and BMI of the groups

	Group 1 (n=44) Median (IQR)	Group 2 (n=44) Median (IQR)	p values
Age (years)	22.5 (20-26)	23 (21-28)	0.51
BMI (kg/m2)	24.1 (21.4-25.2)	22.6 (20.4-24.5)	0.18

BMI: Body mass index, IQR: Interquartile range

The hormonal parameters, glucose metabolism parameters, serum AMH, and vitamin D levels, and clinical signs and symptoms were determined, and the results were shown in **Table 2**. The levels of serum AMH were found statistically significantly higher in Group 1. The levels of vitamin D were found low in both groups. There was no statistically significant difference between the two groups in vitamin D levels. However, the median vitamin D level was slightly higher in the PCOS group unexpectedly (p=0.93).

Table 2: The hormonal and metabolic parameters of the groups

	Group 1 (n=44) Median (IQR)	Group 2 (n=44) Median (IQR)	p values
FSH (mIU/ml)†	5.95 ± 2.06	6.54 ± 1.92	0.16
LH (mIU/ml)	6.65 (4.3-10.65)	3.6 (2.6-6.1)	**<0.001***
E2 (pg/ml)	43.13 (31.2-67.5)	43 (30.2-64.01)	0.71
PRL (ng/ml)	10 (7.5-13.7)	12.05 (7.3-15.2)	0.39
TSH (µIU/ml)	1.56 (1.17-2.3)	1.6 (1.2-2.2)	0.90
DHEA-S (µg/dl)†	244.4 ± 84.28	204.68 ± 95.83	**0.042***
17-OH-PROG (nmol/l)	0.8 (0.57-1.2)	0.7 (0.43-1.1)	0.33
F testosterone (ng/dl)	1.14 (0.84-1.59)	1.2 (0.88-1.5)	0.78
T testosterone (ng/dl)	30.7 (0.6-46.7)	13.6(0.5-30.9)	**0.04***
LH/FSH	1.3 (0.7-2)	0.58 (0.39-1.05)	**<0.001***
Fasting glucose (mg/dl)†	87.25 ± 8.25	88.59 ± 7.33	0.42
Postprandial glucose (mg/dl)	92 (87.3-98)	86.5 (80-94.7)	**0.02***
Insulin (µIU/ml)	8.5 (6.5-17.9)	8.4 (5.4-12.8)	0.1
HbA1C (%)	5.4 (5.2-5.7)	5.4 (5.2-5.6)	0.74
HOMA-IR	1.9 (1.4-3.8)	1.8 (1.09-2.7)	0.12
Vitamin D	12.1 (5.8-20.3)	11.6 (8.2-17.4)	0.93
AMH	5.9 (3.9-8.7)	2.7 (1.8-3.9)	**<0.001***
Hirsutism scores	10 (6-12)	3 (0.5-5)	**<0.001***

*Statistically significant
†Normally distributed variables according to Kolmogorov-Smirnov test (mean± SD)
DHEA-S: Dehydroepiandrosterone sulphate, E2: Estradiol, F testosterone: Free testosterone, FSH: Follicle-stimulating hormone, HOMA-IR: Homeostatic model assessment for insulin resistance, IQR: interquartile range, LH: Luteinizing-hormone, PRL: Prolactine, T testosterone: Total testosterone, TSH: Thyroid-stimulating hormone, 17-OH-PROG: 17-hydroxyprogesterone

There were positive correlations between AMH levels and LH/FSH ratio (p<0.001), hirsutism scores (p=0.001), HOMA-IR (p=0.03) and fasting insulin (p=0.03) (**Table 3**). There were no correlations between AMH and vitamin D, BMI, HbA1C, fasting glucose, postprandial glucose, total testosterone, free testosterone, estradiol levels. In conclusion, the patients with higher AMH levels were more hyperandrogenic compared to the patients with PCOS who have lower AMH levels.

Table 3: Correlations with anti-mullerian hormone

	R	p values
Hirsutism score	0.345	**0.001**
LH/FSH ratio	0.484	**0.000**
HOMA-IR	0.229	**0.032**
Insulin	0.228	**0.033**

FSH: Follicle-stimulating hormone, HOMA-IR: Homeostatic model assessement for insulin resistance, LH: Luteinizing hormone

There was a negative correlation between vitamin D and HOMA-IR. The difference was not statistically significant; however, the p-value was close to the value of 0.05 (p=0.057). There was no statistically significant correlation between serum AMH and serum vitamin D levels.

Discussion

It has been hypothesized that AMH is positively correlated with serum androgens (14). While the severity of hyperandrogenism is correlated with the severity of ovulatory disturbance, hyperandrogenism is suspected to increase the AMH production by promoting an excess of small growing follicles and granulosa cell proliferation (19). In our study, there was a positive correlation between the serum AMH levels and hirsutism and LH/FSH ratio.

AMH levels have a strong correlation with the number of antral follicles, so it has been suggested that serum AMH levels can be used instead of the antral follicle count for the diagnosis of PCOS, and can be integrated into the Rotterdam Criteria (9). Measuring AMH levels in the serum is a more standardized method compared to antral follicle count because the counting process may give different results depending on clinicians' experience, the resolution of the ultrasound machine, or external conditions. Several studies have been performed to assess the use of serum AMH as a diagnostic marker for PCOS and to determine an optimal threshold, but there is still no consensus on the cut-off level of serum AMH. A meta-analysis of these studies shows a cut-off value of 4.7 ng/ml with a sensitivity of 79.4% and a specificity of 82.8% (9). In our study, the mean ages were 22.5 and 23 among the groups, respectively, and it is so close to the age of 25, which is the peak age of AMH (20). Also, in this study, the minimum AMH level was 2.4 ng/ml, the maximum level was 27 ng/ml, and the median level was 5.9 (interquartile range = 3.9-8.7) ng/ml in PCOS patients.

Hyperinsulinaemia was detected more frequently in anovulatory women compared to ovulatory women, and a direct correlation was found between the serum AMH levels and insulin insensitivity (21). Tokmak et al. studied the association between serum AMH levels and IR in non-obese adolescent females with PCOS either with IR or without IR. They reported that there was a significant positive correlation between serum AMH and HOMA-IR levels in PCOS patients (22). We also found similar results regarding the correlation between AMH and HOMA-IR (p=0.03).

There are many studies about the association between vitamin D levels and various PCOS symptoms such as insulin resistance, obesity, infertility, and hirsutism (2,23). There are very different results about the association between vitamin D levels and PCOS. Vitamin D deficiency is also common in the general population in many parts of the world, with 10-60% of adults having less than 20 ng/ml (24). In our study, the median value of vitamin D levels in PCOS patients was 12.1 (5.8-20.3) ng/ml, and in the control group it was 11.6 (8.2-17.4) ng/ml, and although the difference was not statistically significant, PCOS patients had higher serum vitamin D levels. However, it was noteworthy that the median vitamin D levels were low in both groups.

Muscogiuri et al. reported that vitamin D levels were lower in obese PCOS patients than non-obese PCOS patients (25). The high prevalence of vitamin D deficiency in women with PCOS may be related to obesity since vitamin D is fat-soluble, and a higher proportion of vitamin D is sequestered in adipose tissue in obesity. This situation may lower bioavailability (4,25). On the other hand, dietary preferences may differ between obese and non-obese individuals.

The exact mechanism underlying the association of vitamin D and insulin resistance is not known yet. The biologically active form of vitamin D, 1.25-dihydroxy vitamin D (1.25OHD), may increase insulin action by stimulating insulin synthesis and release, increasing insulin receptor expression or suppression of the proinflammatory cytokines that are believed to mediate insulin resistance (26). However, Muscogiuri et al. found that vitamin D deficiency was not related to the presence of insulin resistance, but was related to the presence of obesity (25). Hahn et al. reported that lower levels of 1.25OHD were associated with insulin resistance and obesity (23). However, the results might have been affected by obesity in this study. Also, Wehr et al. reported that the level of 1.25OHD was a significant and independent predictor for HOMA-IR and BMI by using multivariate regression analysis (2). In our study, we found a negative correlation between vitamin D levels and HOMA-IR.

There are some observational studies about the relationship between vitamin D levels and hyperandrogenism. Glintborg et al. reported that 1.25OHD levels were lower in hirsute women

compared to BMI-matched control women (27). In our study, there was no statistically significant correlation between vitamin D and hirsutism or hyperandrogenism. Ardabili et al. studied with 50 patients with PCOS and vitamin D deficiency, however, they did not report a difference for fasting serum insulin and glucose levels and HOMA-IR after vitamin D supplementation (28).

In this study, we tried to determine whether there is a relationship between the serum AMH and vitamin D levels and PCOS clinical signs and symptoms, and the severity of the disease. In conclusion, the patients with higher AMH levels were more hyperandrogenic compared to the patients with PCOS who had lower AMH levels. Besides, the levels of vitamin D were found low in both groups, and there was no statistically significant difference between the two groups in vitamin D levels. Also, further studies are needed to explain and demonstrate the associations between these parameters.

Declaration of Interest

The authors reported no conflict of interest and declared that this study received no financial support.

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