

Vitamin D deficiency in women with polycystic ovary syndrome

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Objective: To investigate: the prevalence of vitamin D deficiency in Korean women with polycystic ovary syndrome (PCOS), and the relationship between vitamin D status and clinical or metabolic features in this group.

Methods: We recruited 38 women with PCOS using the Rotterdam criteria. A total of 109 premenopausal control women were matched with patients based on age and body mass index. Serum 25-hydroxy vitamin D concentrations less than 20 ng/mL were classified as frank vitamin D deficiency. Since vitamin D may play a significant role in metabolic disturbances in women with PCOS, correlations between clinical or metabolic parameters and vitamin D status were analyzed separately in patients and controls.

Results: Women with PCOS showed no differences in the level of 25-hydroxy vitamin D (19.6 ± 6.6 ng/mL in patients vs. 20.1 ± 7.4 ng/mL in controls, respectively, $p = 0.696$) or prevalence of vitamin D deficiency (57.9% in patients vs. 56.5% in controls, respectively, $p = 0.880$). In addition, we did not find any correlations between serum vitamin D level and clinical or metabolic profiles in either PCOS patients or controls.

Conclusion: Our study found no differences in the absolute level of serum vitamin D between PCOS patients and matched controls. Prevalence of vitamin D deficiency was equally common among both patients and controls. Additionally, we did not find any correlations between serum vitamin D level and clinical or metabolic profiles, suggesting that the role of vitamin D in the pathogenesis of PCOS is not yet clear.

Keywords: Insulin resistance; Polycystic ovary syndrome; Vitamin D

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age, and insulin resistance is a core patho-

physiology of this syndrome. Insulin resistance increases the risk of type 2 diabetes (T2DM); therefore, it may be the common link between PCOS and T2DM.

There is increasing evidence that vitamin D affects insulin and glucose metabolism, and a low vitamin D status is suspected to be a risk factor for impaired glucose tolerance, insulin resistance and T2DM [1-5]. While the mechanisms by which vitamin D could alter the risk of insulin resistance or T2DM are not yet clear, there are several studies that report relevant findings. First, a low vitamin D concentration results in an elevated serum level of parathyroid hormone (PTH), and increased PTH concentrations are suspected to be involved in glucose metabolism and decreased insulin sensitivity [6-8]. Second, vitamin D may stimulate the expression of insulin receptors and thereby enhance insulin responsiveness for glucose transport [9,10]. Further-

Received: May 15, 2014 · Revised: Jun 3, 2014 · Accepted: Jun 6, 2014

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*This study was supported by Grant No. 04-2011-0690 from the SNUH Research Fund.

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more, vitamin D and its vitamin D receptor complex regulate over 300 genes, including genes related to glucose metabolism [11].

Considering the above association between vitamin D status and insulin or glucose metabolism, many studies have investigated vitamin D status in women with PCOS. Although there is no consensus on whether or not serum vitamin D levels are different between women with and without PCOS, an inverse association between vitamin D status and metabolic disturbances has been reported in PCOS patients [12–24]. Furthermore, vitamin D receptor gene polymorphism has been associated with an increased risk of PCOS or related metabolic and endocrine phenotypes [19,20,23], suggesting the role of vitamin D in the pathogenesis of PCOS. However, most studies have focused on Caucasian populations, and studies in Asian populations are scarce. In the current study, we examined whether a low vitamin D status can be observed in Korean women with PCOS. In addition, we investigated the relationship between serum vitamin D concentration and metabolic or clinical profiles of PCOS.

Methods

1. Subjects

We recruited 38 women (18–40 years) with PCOS using the Rotterdam criteria [25]. Hirsutism was assessed using the modified Ferriman and Gallwey score (mF-G score) system, and clinical hyperandrogenism (HA) was defined as an mF-G score of 6 or greater [26]. Biochemical HA was defined as follows: total testosterone (T) > 0.68 ng/mL, free T > 1.72 pg/mL, and free androgen index (FAI) > 5.36 [27]. All women with PCOS were screened to exclude hyperprolactinemia and thyroid dysfunction. Serum 17-hydroxyprogesterone (OHP) was also measured, and if the serum 17-OHP level was greater than 2 ng/mL, a repeat test was performed in the early morning during the follicular phase. Patients who showed continuous elevation of 17-OHP were excluded from the study.

A total of 109 premenopausal women were matched with patients based on age (± 2.0 years) and body mass index (BMI) (± 1.0 kg/m²), and the match ratio was one to three. The women in the control group visited Seoul National University Hospital as part of a group check-up for work, and lacked specific health problems. All controls had regular (21–35 day) menstrual cycles, an mF-G score < 6, and all received a transvaginal or transrectal pelvic ultrasound examination to evaluate ovarian morphology and were excluded if polycystic ovary (PCO) morphology was identified.

No PCOS patients or controls had taken any medications, including vitamin D supplements, combined oral contraceptives, lipid-lowering agents or insulin sensitizing agents. The Institutional Review Board (IRB) for human research at Seoul National University Hospital approved this project (IRB number: H-1108-065-373), and written in-

formed consent was obtained from each woman.

2. Clinical and biochemical measurements

Clinical variables, such as body weight, height, waist circumference (WC), and blood pressure were assessed in all subjects. Body fat mass and visceral fat areas were measured by eight-polar bioelectrical impedance analysis using InBody 770 (Biospace, Seoul, Korea) according to the manufacturer's instructions. Using radioimmunoassay (RIA) (Siemens, Los Angeles, CA, USA), serum levels of total T, free T and sex hormone-binding globulin (SHBG) were measured in all PCOS patients and in a subset of controls (n = 39) whose blood samples were taken during the follicular phase of the menstrual cycle. The FAI was calculated as total T/SHBG \times 100, and the testosterone values were converted from nanograms per milliliter to nanomoles per liter using 1 ng/mL = 3.467 nmol/L for the index, as proposed by the manufacturer. The intra- and inter-assay coefficients of variation (CVs) were 4.0% to 11.0% and 5.9% to 12.0% for total T, and 4.0% to 17.0% and 8.0% to 18.3% for free T, respectively.

In all subjects, after a 12 hours overnight fast, serum calcium, glucose, and lipid levels were measured (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Circulating highly sensitive C-reactive protein was measured using a latex turbidimetric immunoassay with a sensitivity of 0.01 mg/dL (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Fasting insulin levels were measured using RIA (BioSource Europe S.A., Nivelles, Belgium), and the homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as glucose (mg/dL) \times insulin (μ U/mL)/405.

Vitamin D level was defined as a serum level of 25-hydroxycholecalciferol [25-(OH)D₃], and was measured by RIA using a 25-(OH)D iodine-125 radioimmunoassay kit (DiaSorin, Stillwater, Minnesota, USA) and a 1470 WIZARD gamma-counter (PerkinElmer, Turku, Finland). Vitamin D insufficiency was defined as a 25-(OH)D₃ concentration of < 30 ng/mL, and frank vitamin D deficiency was defined as a 25(OH)D₃ concentration of < 20 ng/mL [28].

3. Statistical analysis

Deviation of the data from a normal distribution was examined through visual inspection of quantile-normal plots and/or the Shapiro-Wilk test of normality. The data are shown as the mean \pm SD or median value with the range. If a Gaussian distribution was achieved by natural logarithmic or square root transformation, the data are shown as the geometric mean and 95% confidence intervals (95% CI).

Continuous variables were compared using Student's *t*-test, and the prevalence of vitamin D deficiency was compared using the chi-squared test or Fisher's exact test. Since vitamin D may play a significant role in metabolic or hormonal disturbances in women with PCOS [13,14,17,24,29], correlations between clinical or biochemical param-

eters and vitamin D status were analyzed separately in patients and controls using linear regression models. All data analyses were performed using SPSS software (ver. 21.0, IBM Corp., Armonk, NY, USA), and statistical significance was set at a two-tailed *p*-value of < 0.05.

Results

The clinical and biochemical profiles of the subjects are shown in Table 1. PCOS patients had higher hirsutism scores and serum androgen levels by definition, and there were significant differences in the levels of fasting insulin and HOMA-IR between women with PCOS and matched controls.

Women with PCOS showed increased serum levels of calcium compared with matched controls, but they showed no differences in the

levels of 25-(OH)D₃ or prevalence of vitamin D deficiency (< 20 ng/mL) (Table 1). In addition, we did not find any correlations between serum vitamin D level and clinical or metabolic profiles in both PCOS patients and controls (Table 2). Vitamin D insufficiency was observed in the majority of the subjects (92.1% of patients and 87.0% of the controls Table 1).

Discussion

This study focuses on the serum levels of vitamin D and the prevalence of vitamin D deficiency in patients with PCOS. Our study found no differences in the absolute level of serum vitamin D or prevalence of vitamin D deficiency between women with PCOS and matched controls. Additionally, we did not find any correlations between se-

Table 1. Clinical and biochemical features of PCOS patients and matched controls

	PCOS (n = 38)	Controls (n = 109)	<i>p</i> -value ^{a)}
Clinical parameters			
Age (yr)	34.1 ± 4.6	33.2 ± 3.8	0.207
BMI (kg/m ²)	20.1 ± 3.0	19.9 ± 2.5	0.692
WC (cm)	73.3 ± 7.7	73.8 ± 6.9	0.705
Hirsutism score (median [range])	5 (0–19)	0 (0–5)	< 0.001
SBP (mm Hg)	104.8 ± 11.0	103.3 ± 10.0	0.444
DBP (mm Hg)	67.0 ± 8.1	65.7 ± 8.3	0.406
Body fat mass (kg)	13.7 ± 4.9	13.4 ± 4.6	0.774
Visceral fat area (cm ²)	53.9 ± 22.4	51.7 ± 19.0	0.551
Biochemical parameters			
Total T (ng/mL)	0.35 (0.32, 0.38)	0.26 (0.24, 0.28) (n = 39)	< 0.001
Free T (pg/mL)	1.04 (0.87, 1.21)	0.56 (0.48, 0.64) (n = 39)	< 0.001
SHBG (nmol/L)	36.7 (34.6, 38.8)	59.8 (58.1, 61.5) (n = 39)	< 0.001
FAI	3.0 (2.6, 3.4)	1.4 (1.1, 1.7) (n = 39)	< 0.001
LH (IU/L)	7.3 (6.7, 8.0)	Not checked	(-)
FSH (IU/L)	4.6 (4.3, 4.9)	Not checked	(-)
E ₂ (pg/mL)	62.2 ± 9.1	Not checked	(-)
Fasting glucose (mg/dL)	88.6 ± 8.9	87.0 ± 6.6	0.402
Fasting insulin (μU/mL)	8.6 (6.7, 10.5)	6.2 (4.5, 7.9)	< 0.001
HOMA -IR	1.88 (1.52, 2.20)	1.31 (1.24, 1.40)	< 0.001
Total C (mg/dL)	183.1 ± 26.8	175.9 ± 24.7	0.134
TG (mg/dL)	76.2 (74.6, 77.8)	66.4 (65.0, 67.8)	0.158
HDL-C (mg/dL)	63.2 ± 11.3	59.1 ± 11.7	0.061
LDL-C (mg/dL)	105.0 ± 26.0	104.0 ± 23.3	0.822
A1C (%)	5.38 ± 0.22	5.40 ± 0.22	0.587
hs-CRP (mg/dL)	0.02 (0.00–0.66)	0.02 (0.00–1.38)	0.809
Serum calcium (mg/dL)	9.18 ± 0.29	9.03 ± 0.30	0.006
25-(OH) vitamin D ₃ (ng/mL)	19.6 ± 6.6	20.1 ± 7.4	0.696
Prevalence of vitamin D insufficiency (< 30 ng/mL) (n [%])	92.1 (35/38)	87.0 (94/108)	0.560
Prevalence of vitamin D deficiency (< 20 ng/mL) (n [%])	57.9 (22/38)	56.5 (61/108)	0.880

Values are presented as mean ± SD or geometric mean and 95% confidence interval. *p*-values are indicated for the differences between groups, as analyzed using the Student's *t*, chi-squared test or Fisher's exact test.

PCOS, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; T, testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E₂, estradiol; HOMA-IR, homeostatic model assessment for insulin resistance; C, cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; A1C, hemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein; 25-(OH) Vitamin D₃, 25-hydroxy vitamin D₃.

^{a)}PCOS patients versus controls.

Table 2. Correlation of 25-(OH) vitamin D₃ levels with clinical and biochemical parameters

	PCOS		Controls	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Clinical parameters				
Age (yr)	0.110	0.510	-0.096	0.321
BMI (kg/m ²)	-0.049	0.770	0.088	0.363
WC (cm)	0.012	0.942	0.075	0.438
SBP (mm Hg)	-0.057	0.735	0.054	0.577
DBP (mm Hg)	0.062	0.710	0.061	0.531
Body fat mass (kg)	-0.150	0.370	0.093	0.337
Visceral fat area (cm ²)	-0.099	0.556	0.036	0.711
Biochemical parameters				
Total T (ng/mL)	-0.120	0.235	-0.135	0.562
Free T (pg/mL)	-0.011	0.265	0.022	0.702
SHBG (nmol/L)	0.168	0.321	0.120	0.573
FAI	-0.062	0.262	-0.023	0.901
Fasting glucose (mg/dL)	-0.180	0.280	-0.149	0.123
Fasting insulin (μU/mL)	0.009	0.902	-0.201	0.296
HOMA-IR	-0.015	0.825	-0.200	0.256
Total C (mg/dL)	0.212	0.200	-0.108	0.265
TG (mg/dL)	-0.015	0.930	-0.092	0.345
HDL-C (mg/dL)	0.114	0.494	0.017	0.859
LDL-C (mg/dL)	0.175	0.294	-0.080	0.410
A1C (%)	-0.084	0.518	-0.131	0.175
hs-CRP (mg/dL)	-0.151	0.393	-0.025	0.805
Serum calcium (mg/dL)	-0.166	0.318	0.031	0.753

Correlation between variables was determined using linear regression analysis. 25-(OH) Vitamin D₃, 25-hydroxy vitamin D₃; PCOS, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; T, testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; HOMA-IR, homeostatic model assessment for insulin resistance; C, cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; A1C, hemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein.

rum vitamin D and hormonal or metabolic profiles in either PCOS patients or controls. Our findings suggest that the role of vitamin D in the pathogenesis of PCOS is not yet clear.

Although Wehr et al. [20] reported lower serum vitamin D levels in a large number of women with PCOS (n = 545) compared to controls (n = 145) (25.7 vs. 32.0 ng/mL, respectively), a substantial number of studies suggest that serum vitamin D levels are similar in women with and without PCOS [12,17,30]. In fact, there has been one report that women with PCOS have significantly higher vitamin D levels compared to control women with similar age and BMI [15]. Thus, there is inconsistency in the literature about whether vitamin D levels are similar between women with and without PCOS.

In previous studies, average serum vitamin D levels in women with PCOS were reported to be between 11 ng/mL and 31 ng/mL, with the majority having mean values < 20 ng/mL [12,13,15-17,20,22,29,31-33]. In our study, the mean 25-(OH)D₃ level in women with PCOS was also < 20 ng/mL (19.6 ± 6.6 ng/mL), and vitamin D deficiency

(lower than 20 ng/mL) was observed in 57.9% of patients. However, vitamin D deficiency is also common in the general population, with 10% to 60% of adults having values lower than 20 ng/mL [34,35]. The control subjects in the current study also showed a high prevalence of vitamin D deficiency (56.5%), with a mean level of 20.1 ng/mL. Thus, we can assume that although there is inconsistency in the literature about whether vitamin D levels are different between women with and without PCOS, vitamin D deficiency is equally common in both groups. In fact, in our study, vitamin D insufficiency (< 30 ng/mL) was observed in the majority of the subjects (92.1% of patients and 87.0% of the controls), which is supported by the findings that more than 90% of the pigmented population of the United States (Blacks, Hispanics, and Asians) suffer from vitamin D insufficiency [28].

Many studies have investigated an association between vitamin D status and hormonal or metabolic features in PCOS. In women with PCOS, a low vitamin D level is thought to be related to metabolic risk factors such as insulin resistance, high total cholesterol, blood pressure, glucose, C-reactive protein, triglycerides, and low high-density lipoprotein (HDL) cholesterol [14,17]. In addition, vitamin D replacement therapy may have a beneficial effect on insulin resistance or fasting and on stimulated glucose and triglycerides levels in women with PCOS [16,33]. Furthermore, several studies have identified relationships between low vitamin D status and measures of hyperandrogenism such as SHBG, the degree of hirsutism, FAI, total T and dehydroepiandrosterone sulphate [13,14,17,29]. However, we did not find any correlations between serum vitamin D and hormonal or metabolic profiles in either PCOS patients or controls.

In the current study, a higher serum concentration of calcium was seen in women with PCOS. Although some reports found no differences [15,17], lower serum calcium concentrations in PCOS have also been reported [13,21,29]. Experimental studies show that an increase in intracellular free calcium is essential for meiotic resumption by mouse oocytes [36], and it has been suggested that disordered calcium regulation may in part be responsible for disorders of oocyte development such as PCOS [31]. It is not clear from our study why women with PCOS showed higher calcium levels than controls, and this needs to be investigated in further studies.

Although our study found no differences in the absolute level of serum vitamin D or prevalence of vitamin D deficiency between PCOS women and matched controls, the results need to be interpreted with caution. First, vitamin D deficiency may be a universal phenomenon across PCOS patients and controls. Second, inverse associations between obesity (BMI, body fat and waist measurements) and serum vitamin D levels have been reported in many studies [12-14,17,22,29]. As vitamin D is fat soluble, a higher proportion of vitamin D may be sequestered in adipose tissue in obese individuals, which might lower serum levels. In our study, patients and controls were matched by

BMI, thus there were no differences in BMI, WC, or body and visceral fat masses between the two groups. Thus, it is possible that a similar degree of obesity might obscure the detection of any differences in serum levels of vitamin D between patients and controls. Third, a potential limitation of the present study is the modest sample size in the PCOS group, which precludes drawing strong conclusions. Finally, we did not evaluate the presence of other potential confounding factors, such as outdoor times or dietary patterns which could affect the serum vitamin D levels.

In summary, we found no difference in the absolute level of serum vitamin D or prevalence of vitamin D deficiency between women with PCOS and matched controls. Additionally, we did not find any correlation between serum vitamin D and hormonal or metabolic profiles in either PCOS patients or controls. Although our findings suggest that the role of vitamin D in the pathogenesis of PCOS is not yet clear, vitamin D deficiency is a common finding among PCOS patients and controls. Finally, the potential relationship between vitamin D and PCOS requires further investigation, since vitamin D deficiency has been continuously proposed to increase the risk of insulin resistance and T2DM, which is also a core pathophysiology of PCOS.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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