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Intrinsic factors rather than vitamin D deficiency are related to insulin resistance in lean women with polycystic ovary syndrome

S. SAHIN[1], M. EROGLU[1], S. SELCUK[1], L. TURKGELDI[1], S. KOZALI[1], S. DAVUTOGLU[2], M. MUHCU[1,3]

[1]Zeynep Kamil Woman's and Children's Disease Training and Research Hospital, Istanbul, Turkey
[2]Medikalpark Hospital, Istanbul, Turkey
[3]GATA Haydarpasa Training Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey

Abstract. – OBJECTIVE: To investigate the correlation between insulin resistance (IR) and serum 25-OH-Vit D concentrations and hormonal parameters in lean women with polycystic ovary syndrome (PCOS).

PATIENTS AND METHODS: 50 lean women with PCOS and 40 body mass index (BMI) matched controls were compared in terms of fasting insulin and glucose, homeostatic model assessment insulin resistance (HOMA-IR), 25-OH-Vit D, high sensitivity C-reactive protein (hs-CRP), luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, dehydroepiandrosterone sulfate (DHEA-S), total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides and Ferriman-Gallway (FG) scores. Correlation analyses were performed between HOMA-IR and metabolic and endocrine parameters.

RESULTS: 30% of patients with PCOS demonstrated IR. Levels of 25-OH-Vit D, hsCRP, cholesterol, HDL, LDL, triglyceride and fasting glucose did not differ between the study and control groups. Fasting insulin, HOMA-IR, LH, total testosterone, and DHEA-S levels were higher in PCOS group. HOMA-IR was found to correlate with hs-CRP and total testosterone but not with 25-OH-Vit D levels in lean patients with PCOS.

CONCLUSIONS: An association between 25-OH-Vit D levels and IR is not evident in lean women with PCOS. hs-CRP levels do not indicate to an increased risk of cardiovascular disease in this population of patients. Because a strong association between hyperinsulinemia and hyperandrogenism exists in lean women with PCOS, it is advisable for this population of patients to be screened for metabolic disturbances, especially in whom chronic anovulation and hyperandrogenism are observed together.

Key Words:
Vitamin D, Insulin resistance, Polycystic ovary syndrome, Lean PCOS, C-reactive protein, Hyperandrogenism.

Introduction

Vitamin D is an essential regulator of bone and mineral homeostasis. It acts by inducing calcium and phosphorus absorption in the intestines and also has a direct effect on bone formation. The active form of vitamin D3, 1,25-dihydroxyvitamin D3, is derived by two-step hydroxylations of cholecalciferol in the liver and kidneys. It exerts its effects through the Vitamin D receptor[1]. Recent studies have demonstrated hypovitaminosis D to be associated with an increased likelihood of developing metabolic disorders (i.e. insulin resistance, diabetes mellitus)[2]. Vitamin D deficiency has also been demonstrated in patients with polycystic ovary syndrome (PCOS)[3]. Approximately 5-10% of women of reproductive age suffer from this hormonal disorder characterized by oligo-anovulation and clinical or biochemical signs of hyperandrogenism. Obese patients with PCOS have been shown to have lower serum levels of 25-hydroxyvitamin D (25-OH-Vit D) than non-obese women with PCOS and vitamin D deficiency has been suggested to have a role in the development of insulin resistance (IR) and impaired glucose tolerance in such patients[4]. However, it is not clear whether vitamin D insufficiency is a causative factor for insulin resistance (IR) or whether it is a consequence of obesity in patients with PCOS. Vitamin D deficiency has also been reported in lean women with PCOS, who are reported to be less insulin resistant than their obese counterparts[5,6]. A question that needs to be answered is whether vitamin D is related to IR in lean women with PCOS or whether other factors are responsible for the IR in this subgroup of women. In this study, we aimed to evaluate the correlation between

Corresponding Author: Sadik Sahin, MD; e-mail: drsadiksahin@gmail.com

homeostatic model assessment insulin resistance (HOMA-IR) and serum 25-OH-Vit D concentrations and hormonal parameters in lean women with PCOS.

Patients and Methods

The study subjects were recruited from the outpatient clinics of our tertiary center between March and June 2014. A total of 90 women were included in this study. Of these women, 50 were diagnosed with PCOS according to the Rotterdam criteria[7]. Two of the following three criteria were required to confirm the diagnosis of PCOS: oligo-anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries on ultrasound examination (defined as > 12 antral follicle count in each ovary). Those with body mass index (BMI) < 25 kg/m^2 were included in this study. 40 age- and BMI-matched healthy women were studied as the control group. Patients with congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, and androgen secreting tumors as well as patients using insulin sensitizing drugs, anti-hyperlipidemic drugs, anti-inflammatory drugs and oral contraceptives were excluded. The local Ethics Committee approved the study.

A fasting blood sample was taken on day 2 or 3 of each patient's spontaneous or progestin-induced menstrual cycle. Levels of serum 25-OH-Vit D, insulin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone and dehydroepiandrosterone sulfate (DHEA-S) were measured by chemiflex immunoassay using Architect i2000SR Immunoassay Analyzer (Abbott, Abbott Park, IL, USA). Fasting glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides and highly sensitive C reactive protein (hsCRP) were measured by Architect C16000 (Abbott). The homeostasis model assessment of insulin resistance (HOMA-IR) was used to evaluate insulin resistance, using the following formula: HOMA-IR = (Fasting plasma glucose (mg/dL) × insulin (U/mL)/405)[8]. Patients with HOMA-IR levels above 2.5 were considered to have IR[9]. The degree of hirsutism was evaluated using the Ferriman-Gallwey (FG) score[10].

Statistical Analysis

The GraphPad Prism 6.0 software (GraphPad, San Diego, CA, USA) was used for statistical analyses. Descriptive results of continuous variables are expressed as mean±standard deviation. Student's *t* test was used to compare data from PCOS and control groups. Correlation analyses were investigated using the Pearson test. $p < 0.05$ was accepted as significant.

Results

Approximately 30% of patients with PCOS had oligo-anovulation characterized by a delay or absence of regular menstruation. Fifteen out of 50 patients had HOMA-IR levels above 2.5 and were considered to have insulin resistance. The metabolic and hormonal characteristics of women with PCOS and the control group are presented in Table I. LH, total testosterone and DHEA-S levels were significantly higher in women with PCOS than the control group ($p < 0.0001$, $p < 0.0001$ and $p < 0.01$, respectively). Similarly, the FG scores were higher in the PCOS group when compared with the control group. Fasting insulin and HOMA-IR levels were higher in patients with PCOS than healthy women ($p = 0.02$ and $p = 0.04$, respectively). No significant differences were found between the two groups in terms of age, BMI and serum levels of cholesterol, HDL, LDL, triglyceride, glucose, hsCRP, FSH and estradiol.

Correlation of HOMA-IR with metabolic and endocrine parameters and 25-OH-Vit D levels in the PCOS and control groups are given in Table II. No significant correlation was found between HOMA-IR and 25-OH-Vit D levels (Figure 1). Although the mean hsCRP levels were found to be similar in the two groups, correlation analysis revealed a positive correlation between HOMA-IR and hsCRP levels in the PCOS group ($p = 0.03$). The FG score, LH and total testosterone (Figure 2) levels were also found to be positively correlated with HOMA-IR in the PCOS group ($p = 0.001$, $p = 0.01$ and $p = 0.03$, respectively).

Discussion

In this study, we evaluated IR in relation to vitamin D and hs-CRP levels and metabolic and hormonal parameters in lean patients with PCOS and BMI matched controls. We found 25-OH-Vit D levels to be similar in lean patients with PCOS and healthy patients. Furthermore, levels of 25-OH-Vit D were not found to correlate with IR in

Table I. Metabolic and hormonal characteristics of PCOS and control groups.

Variable	Lean PCOS (n=50)	Control (n=40)	*p* value
Age (years)	27.30 ± 0.54	29.05 ± 0.79	0.07
BMI (kg/m^2)	24.66 ± 0.38	24.05 ± 0.55	0.35
FG score	8.50 ± 0.55	3.80 ± 0.21	< 0.0001*
Cholesterol (mg/dl)	172.5 ± 5.07	165.3 ± 5.98	0.35
HDL (mg/dl)	48.08 ± 1.33	45.58 ± 1.59	0.22
LDL (mg/dl)	105.0 ± 4.10	103.1 ± 4.60	0.74
Triglyceride (mg/dl)	138.7 ± 44.05	102.2 ± 7.56	0.46
Glucose (mg/dl)	86.68 ± 1.379	90.13 ± 2.30	0.18
Insulin (µU/ml)	9.79 ± 0.68	7.68 ± 0.81	0.02*
HOMA-IR	2.13 ± 0.16	1.69 ± 0.11	0.04*
hsCRP (mg/dl)	0.28 ± 0.05	0.24 ± 0.03	0.53
25-OH-Vit D (ng/ml)	12.23 ± 0.92	14.37 ± 2.73	0.49
LH (mIU/ml)	8.30 ± 0.78	4.34 ± 0.22	< 0.0001*
FSH (mIU/ml)	5.93 ± 0.81	6.19 ± 0.55	0.80
Estradiol (pg/ml)	42.95 ± 2.34	50.39 ± 4.41	0.12
T. Testosterone (ng/ml)	0.45 ± 0.02	0.31 ± 0.01	< 0.0001*
DHEA-SO4 (µg/ml)	305.3 ± 31.21	226.0 ± 13.95	0.01*

FG score: Ferriman-Gallwey.

patients with PCOS. IR, however, was found to correlate positively with hsCRP, LH and total testosterone levels in lean women with PCOS.

The prevalence of IR was higher in patients with PCOS than the control group consistent with previous reports[11-13]. Approximately 1/3rd of lean patients with PCOS demonstrated IR. Both fasting insulin and HOMA-IR values were higher in this population of patients when compared with the control group. On the other hand, BMI was not found to correlate with IR in lean PCOS women. This may be related to the nature of PCOS itself in which intrinsic mechanisms and genetic predisposition may play an important role in the pathogenesis of IR, independent of obesity. Nevertheless, obesity is a common feature of PCOS affecting 50-80% of patients with this disorder[14]. Increased BMI has been shown to induce impaired glucose tolerance and elevation in circulating insulin levels, worsening IR[12]. Rotterdam Consensus advised weight loss as a first line treatment strategy for obese PCOS women to alleviate metabolic disturbances and menstrual irregularities[7]. However, there is no consensus on whether lean women with PCOS should be treated with insulin lowering medications (i.e. metformin) for IR.

Table II. Correlation of HOMA-IR with metabolic, endocrine parameters and 25-OH-Vit D levels in PCOS and control groups.

Variable	Lean PCOS		Control	
	r	*p*	r	*p*
BMI	0.16	0.26	0.18	0.19
FG score	0.46	0.001*	0.13	0.43
25-OH-Vit D	-0.13	0.40	-0.12	0.49
LH	0.35	0.01*	-0.13	0.41
T. Testosterone	0.30	0.03*	0.31	0.06
DHEA-SO	0.14	0.64	0.06	0.74
FSH	-0.03	0.79	-0.09	0.58
Estradiol	-0.04	0.78	-0.15	0.36
hsCRP	0.29	0.03*	-0.09	0.57
Cholesterol	0.05	0.73	-0.06	0.71
HDL	0.02	0.91	-0.07	0.96
LDL	-0.05	0.71	0.04	0.78
Triglyceride	0.16	0.25	-0.01	0.95

r: correlation coefficient; FG score: Ferriman-Gallwey.

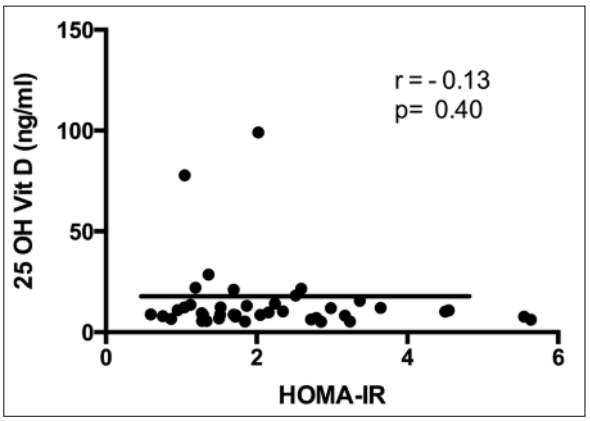

Figure 1. Correlation between HOMA-IR and 25-OH-Vit D levels in PCOS group.

25-OH-Vit D levels were found to be similar in lean patients with PCOS and healthy women. Although 1/3rd of PCOS patients in the study were found to be insulin resistant, no correlation was found between 25-OH-Vit D levels and HOMA-IR. There are conflicting reports about the relationship between Vitamin D deficiency and IR in women with PCOS[4,15,16]. While some studies have demonstrated a negative correlation between Vitamin D levels and HOMA-IR, others have failed to show any relationship between the two parameters. The discrepancy among previous reports may be due to the lack of a uniform distribution of populations and methodologies used. Moreover, Vitamin D deficiency has been shown to occur more commonly in obese patients with PCOS. A recent study stated that the main determinant of low 25-OH-Vit D levels in women

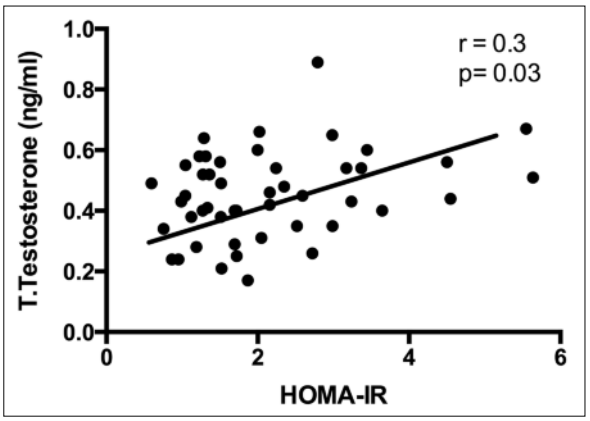

Figure 2. Correlation between HOMA-IR and total testosterone levels in PCOS group.

with PCOS is the degree of adiposity rather than IR[16]. Our results support the findings of this study. Since only lean patients with PCOS were included in the present study, the effect of obesity on vitamin D levels was eliminated. Furthermore, studies investigating the beneficial effects of Vitamin D supplementation on insulin-resistance in women with PCOS have yielded conflicting results[17,18]. Based on these findings, Vitamin D deficiency does not seem to be a causative factor of IR in lean women with PCOS.

Hyperinsulinaemia and hyperandrogenemia have been shown in numerous studies to correlate positively in patients with PCOS[19]. A question of interest is whether hyperinsulinemia is a cause or effect of hyperandrogenism in PCOS. A study by Baillargeon et al[20] showed that metformin treatment decreased testosterone levels in non-obese women with PCOS by reducing insulin levels. In addition, myo-inositol treatment resulted a decrease in the serum levels of insulin and total testosterone and also an improvement of oocyte quality in women with PCOS[21-23]. Hyperinsulinemia, in compensation for IR, is thought to lead to hyperandrogenemia by acting directly on ovarian theca cells to produce androgens and by suppressing sex hormone binding globulin production by the liver. High levels of circulating androgens may in turn aggravate basal IR, further worsening the clinical picture of PCOS[19]. This may explain why metabolic abnormalities are more often encountered in patients with hyperandrogenism and chronic anovulation. In this study, a strong correlation between hyperandrogenism (high FG score, LH and total testosterone levels) and HOMA-IR was found. These findings support the idea that patients with the hyperandrogenic and oligo-anovulatory phenotype of PCOS should be followed-up closely as they may be more likely to develop metabolic syndrome or Type 2 diabetes in the future.

The prevalence of cardiovascular disease is higher in patients with PCOS than healthy individuals since obesity, dyslipidemia, insulin resistance and hypertension are frequently encountered in such patients[24]. It is not known, however, whether PCOS itself is an additional risk factor for CVD, independent of BMI and other components of the metabolic syndrome. Several studies have reported higher levels of C-reactive protein (CRP), a predictor of cardiovascular disease, in patients with PCOS than healthy individuals[25,26]. Tosi et al[27] reported hs-CRP to correlate negatively with insulin sensitivity and positively with

body fat mass and that these two parameters are independent predictors of serum hs-CRP in patients with PCOS. The same group also reported that the independent effect of body fat on hs-CRP levels was lower in lean patients with PCOS. In the present study, no significant difference was observed in serum hs-CRP levels between lean patients with PCOS and the controls. However HOMA-IR levels were found to correlate positively with hsCRP. The lack of a significant difference in hs-CRP between the two groups in the present study may be due to the involvement of only lean patients with PCOS, blunting the effect of body fat mass on serum hs-CRP. Furthermore, serum androgens have been shown to have anti-inflammatory effects in animal models[28], which may have become more evident in this study population.

Conclusions

Vitamin D deficiency is not correlated with IR in lean women with PCOS and intrinsic mechanisms and genetic predisposition seem to be responsible for IR in these patients. Although positively correlated with IR, hs-CRP levels do not indicate to an increased risk of cardiovascular disease in lean PCOS patients. Because a strong association between hyperinsulinemia and hyperandrogenism exists in lean women with PCOS, it is advisable for this population of patients to be screened for metabolic disturbances, especially in whom chronic anovulation and hyperandrogenism.are observed together.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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