Original Communication

25(OH) Vitamin D Levels in Premenopausal Women with Polycystic Ovary Syndrome and/or Obesity

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Abstract: *Background:* Insulin resistance, hyperinsulinemia, and obesity play an important role in development of polycystic ovary syndrome (PCOS). Current evidence suggests that vitamin D (VitD) deficiency may contribute to the disturbance in insulin metabolism and the development of the metabolic syndrome. The *aim* of this study was to investigate VitD levels, measured as 25(OH)D, in Bulgarian women with PCOS and/or obesity. *Materials and methods:* The study included 103 women, divided into three groups – group *1* Obese (n = 33); *group 2* Nonobese PCOS (n = 50), and *group 3* Obese PCOS (n = 20). 25(OH)D levels were measured by electrochemiluminescence immunoassay. *Results:* Almost 2/3 of the women with PCOS and/or obesity appeared to be VitD-deficient. Women with obesity, especially visceral (with or without PCOS), had significantly lower levels of 25(OH)D compared to lean PCOS subjects. Women with and without metabolic syndrome however did not differ significantly in 25(OH)D levels. Women with normal body mass index (BMI) had higher 25(OH)D levels compared to overweight and obese ($p = 0.028$). There was no correlation between 25(OH)D levels and indices of glucose metabolism – fasting blood glucose and immunoreactive insulin (IRI) and after OGTT and HOMA index.

Key words: vitamin D, PCOS, obesity, insulin resistance

Introduction

Vitamin D is thought to play a role in the pathogenesis of metabolic syndrome and type 2 diabetes by affecting insulin metabolism. Some authors found significantly negative correlations of 25-hydroxyvitamin D [25(OH)D] levels with body mass index (BMI), waist circumference, waist-to-hip ratio, systolic and diastolic blood pressure, fasting and stimulated glucose, area under the glucose response curve, fasting insulin, homeostatic model assessment of insulin resistance (HOMA-IR), HOMA-beta, triglycerides, and quotient total cholesterol/high-density lipoprotein (HDL) and positive correlations of 25(OH)D levels with quantitative insulin sensitivity check index (QUICKI) and HDL [1].

Some studies suggest that vitamin D deficiency might be involved in the pathogenesis of insulin resistance and the metabolic syndrome in polycystic ovary syndrome (PCOS) [5,19]. In two studies involving 85 PCOS and 115 control women, and 25 PCOS women and 27 controls, respectively [3,20], vitamin D deficiency was found to be more common in PCOS women than in controls. Severe vitamin D deficiency [25(OH)D < 25 nmol/L] was found in 44.0 % and 11.2 % of subjects in the PCOS and control groups, respectively [3]. Obesity and PCOS are both associated with unfavorable cardiometabolic profile and insulin resistance. In large epidemiological studies, lower serum 25(OH)D levels are associated with a higher prevalence of obesity [2]. Vitamin D deficiency was found to be independently associated with low insulin sensitivity assessed by QUICKI [5]. In another study among the PCOS subjects, 25(OH)D concentrations were negatively correlated with body mass index, C-reactive protein, and free androgen index and positively correlated with QUICKI, high-density lipoprotein cholesterol (HDL-C), and sex hormone-binding globulin. Associations of vitamin D deficiency with QUICKI and HDL-C were independent of body mass index and waist-to-hip ratio. In another study, serum 25(OH)D mean levels were 56.31 % lower in obese PCOS patients [4]. There are some data about an inverse association of 25(OH)D with obesity as well as a BMI-dependent association with insulin resistance [21]. Moreover some studies reported an association of low vitamin D status with insulin resistance only in obese PCOS women [4, 5, 21]. So far, it is not clear whether vitamin D insufficiency results from obesity and/or if obesity is a consequence of vitamin D insufficiency [22].

Some studies find an association of increased total testosterone and dehydroepiandrosterone sulfate (DHEAS) with decreased 25(OH)D concentrations in the obese PCOS patients [4]. In other studies, analysis of vitamin D and biochemical endocrine PCOS features revealed a significant correlation only between 25(OH)D and sex hormone-binding globulin as well as the free androgen index, but not with total testosterone, androstenedione, DHEAS, estradiol, or luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio [5].

There are no data from Bulgaria about vitamin D levels in PCOS. The aim of this study was to investigate 25(OH)D levels in Bulgarian women with PCOS and/or obesity.

Materials and Methods

In the present study were included women with PCOS and/or obesity that met the following inclusion and exclusion criteria.

Inclusion criteria
- premenopausal women aged 18 to 45
- PCOS, diagnosed by ESHRE-ASRM criteria [6] and/or
- obesity (BMI > 30 kg/m^2)

Exclusion criteria
- Pregnancy
- Serious illnesses such as cardiac, renal, or liver insufficiency
- Other endocrine pathology such as type 2 diabetes mellitus, adrenal tumors, hypothyroidism, pituitary tumors, hypogonadism
- Insulin-sensitizing medication (metformin or glitazones) or combined oral contraceptive (COC) use less than 4 months prior to the study

The study was performed in accordance with the guidelines of the Declaration of Helsinki. Women patients signed an informed consent for participation in the study.

The following information for each patient was obtained: General information (name, age); Anthropometric data [height, weight, BMI), waist circumference, hip circumference, waist-to-hip ratio (WHR), waist-to-stature ratio (WSR)]; Body fat was measured by Tanita Professional Analyzer of body composition Model *BC 408*; Obesity was diagnosed at BMI ≥ 30 kg/m^2 [7]; Polycystic ovary syndrome was diagnosed according to the ESHRE-ASRM criteria [6] – two out of the following: 1) oligo/amenorrhea; 2) clinical and/or biochemical hyperandrogenism; and 3) polycystic ovaries at ultrasound examination when all other endocrine causes are excluded.

Results form the oral glucose tolerance test (OGTT) – blood glucose (BG) and immunoreactive insulin (IRI) at 0, 60, and 120 minutes. Homeostasis model assessment (HOMA) index was calculated using the following formula HOMA index = (fasting BG × fasting IRI)/22.5.

Hormones [testosterone, androstenedione, DHEAS, 17-OH-progesterone, estradiol, LH, FSH, thyroid-stimulating hormone (TSH), prolactin].

Metabolic syndrome (MetS) was diagnosed according to the International Diabetes Federation (IDF) [8] and AHA/NHLBI criteria – 3 out of 5 risk factors – increased waist circumference (> 80 cm), increased

TG (>1.7 mmol/L), decreased HDL (<1.3 mmol/L), increased blood pressure (BP) (>130/85 mmHg), and increased fasting blood glucose (>5.5 mmol/L).

25(OH)D measurement

The laboratory tests were performed in the Central Clinical Laboratory of the Alexandrovska University Hospital in Sofia, which is the reference laboratory for the country. The blood for biochemical study was taken after overnight fast and the measurements were performed on the same day. For measuring IRI, testosterone, and DHEAS, electrochemiluminescence (ECLIA) method was performed on an Analytics Elecsys 2010 unit. Androstenedione and 17-OH-progesterone were measured by solid-phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. Estradiol, FSH, and LH were measured by immunochemiluminescent test.

25(OH)D levels were measured by electrochemiluminescence immunoassay (Roche Elecsys 2010 Chemistry Analyzer), based on the competition principle (Vitamin D total, Roche Diagnostics GmbH, D-68305 Mannheim). Precision was determined using pooled human sera and controls according to a modified protocol (EP5-A2) of the Clinical and Laboratory Standards Institute: 2 runs per day in duplicate and each for 21 days (n = 84). Coefficient of variance (CV) was between 1.7 % and 7.5 % for mean values of 25(OH) D between 17.9 mmol/L and 170 mmol/L.

Subjects were categorized by their vitamin D status on the basis of their 25(OH)D levels as follows: group 1 – *optimal* ≥ 75 nmol/L (≥ 30 ng/mL); group 2 – *insufficient* 50 – 74.9 nmol/L (20.0 – 29.9 ng/mL) and group 3 – *deficient* < 49.9 nmol/L (< 20.0 ng/mL) [9].

Statistical methods

The data were processed using the statistical package SPSS 16.0. The level of significance for rejecting the null hypothesis was $p < 0.05$. The following statistical methods were applied: descriptive analysis, variation analysis, Kolmogorov-Smirnov's one-sample non-parametric test, Student's *t*-test for two independent samples, Mann-Whitney's non-parametric test for two independent samples, one-way analysis of variance between-groups ANOVA, and correlation analysis. Data are presented as means ± SD.

Results

One-hundred three women were included in the study. They were divided into three groups: group 1 Obese (n = 33); group 2 Nonobese PCOS (n = 50); and group 3 Obese PCOS (n = 20). Anthropometric characteristics of the subjects are shown in Table I.

The data about 25(OH)D measurements are shown on Table II.

Women with obesity (with or without PCOS) had lower levels of 25(OH)D compared to lean PCOS subjects (Table II). After age adjustment was performed however, no statistical significance was found

Table I: Anthropometric characteristics of the groups.

	Group 1 Obese (n = 33)	Group 2 Nonobese PCOS (n = 50)	Group 3 Obese PCOS (n = 20)
Age (years)	32.0 ± 8.1$^{\wedge\wedge\wedge}$ ($p < 0.001$)	24.0 ± 5.0	25.1 ± 4.8$^{¥¥¥}$ ($p = 0{,}001$)
BMI (kg/m2)	41.2 ± 9.6$^{\wedge\wedge\wedge}$ ($p < 0.001$)	22.6 ± 3.3*** ($p < 0.001$)	36.2 ± 6.1
Waist (cm)	113.2 ± 15.3$^{\wedge\wedge\wedge}$ ($p < 0.001$)	77.3 ± 9.1*** ($p < 0.001$)	104.4 ± 13.5
Hip (cm)	127.1 ± 15.3$^{\wedge\wedge\wedge}$ ($p < 0.001$)	96.9 ± 8.9*** ($p < 0.001$)	118.9 ± 9.5
WHR	0.89 ± 0.08$^{\wedge\wedge\wedge}$ ($p < 0.001$)	0.79 ± 0.06*** ($p < 0.001$)	0.88 ± 0.08
WSR	0.69 ± 0.09$^{\wedge\wedge\wedge}$ ($p < 0.001$)	0.47 ± 0.06*** ($p < 0.001$)	0.64 ± 0.09

$^{\wedge\wedge\wedge}p < 0.001$ between group 1 and group 2;
$^{¥}p < 0.05$; $^{¥¥¥}p < 0.001$ between group 1 and group 3;
$^{*}p < 0.05$; $^{***}p < 0.001$ between group 2 and group 3.

Table II: Results from 25(OH)D measurement.

	Group 1 Obese	Group 2 Lean PCOS	Group 3 Obese PCOS
25(OH)D nmol/l	40.9 ± 23.6	52.3 ± 25.3	40.6 ± 19.9
25(OHOD ≥ 75 nmol/L (optimal)	9.1 %	14 %	10 %
25(OH)D 75 – 50 nmol/L (insufficiency)	18.2 %	26 %	20 %
25(OH)D < 50 nmol/L (deficiency)	72.7 %	60 %	70 %

¥ p < 0.05; between group 1 and group 3;
^ p < 0.005 between group 1 and group 2.

Table III: Correlation coefficient (r) between 25(OH)D and indices of obesity

	Weight	BMI	Waist	WSR	Fat mass	Fat %
25(OH)D	–0.202* (p = 0.042)	–0.228* (p = 0.024)	–0.213* (p = 0.034)	–0.229* (p = 0.022)	–0.163	–0.146*

* p < 0.05

Table IV: Differences in 25(OH)D between patients with and without visceral obesity according to WC and WSR.

	25(OH)D nmol/L
WC ≥ 80 cm	40.8 ± 20.1** (p = 0.004)
WC < 80 cm	57.7 ± 28.1
WSR ≥ 0.5	42.3 ± 23.3* (p = 0.008)
WSR < 0.5	54.1 ± 24.4

* p < 0.05;
** p < 0.01.

between the three groups (p = 0.166). Approximately 2/3 of women in the three groups were VitD-deficient, although the rate of vitD deficiency was lower in the lean PCOS group. 25(OH)D levels showed negative correlation with body weight, BMI, waist circumference, and WSR (Table III). There was no correlation between 25(OH)D levels and indices of glucose metabolism – fasting blood glucose and IRI and after OGTT and HOMA index. A strong negative correlation (r = –0.497, p = 0.015) was observed between 25(OH)D levels and DHEAS levels only in patients with normal weight (BMI < 25 kg/m^2). All correlations were age-adjusted using partial correlation.

Women with visceral obesity had lower levels of 25(OH)D compared to those without (Table IV). Patients with and without metabolic syndrome however did not differ significantly in 25(OH)D levels.

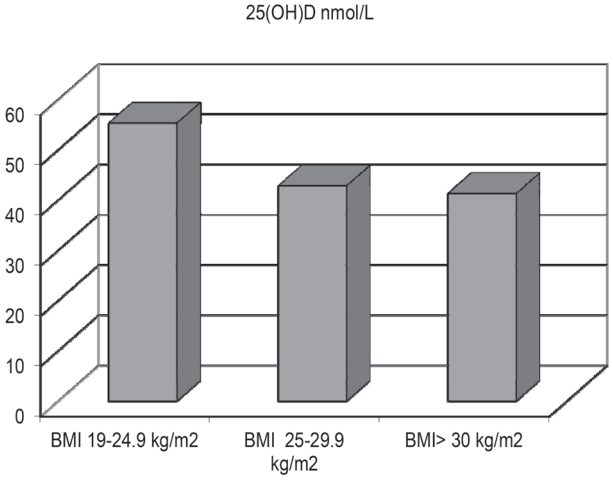

Figure 1: Mean 25(OH)D levels in patients with normal weight, overweight and obesity according to BMI (p = 0.028).

Women with normal BMI (19 – 25 kg/m^2), overweight (BMI 25 – 30 kg/m^2), and obesity (BMI > 30 kg/m^2) differed significantly in 25(OH)D levels (p = 0.028) as shown on Figure 1.

There were no differences in 25(OH)D levels between women with different PCOS phenotype, with and without insulin resistance based on a variety of criteria, with and without arterial hypertension, dyslipidemia, hyperandrogenemia, menstrual disturbances, and obstructive sleep apnea.

The rate of insulin resistance, defined as stimulated IRI > 100 mU/L during OGTT, in patients with

Table V: Rate of insulin resistance (IRI > 100 mU/L during OGTT) in patients with different vitD status.

VitD status	Not insulin-resistant	Insulin-resistant
Optimal (≥75 nmol/L)	91.7 %	8.3 %*
Insufficiency (75 – 50 nmol/L)	47.8 %	52.2 %*
Deficiency (< 50 nmol/L)	63.2 %	36.8 %*

* $p < 0.05$

optimal vitD levels is much lower compared to the other two groups with insufficiency and deficiency (8.3 % vs. 52.2 % and 36.8 %, respectively) and the total group (36.9 %). When using different indices of insulin resistance (HOMA index >2.5 or fasting IRI >20 mU/L), there is no statistical significant difference between the rates of insulin resistance between patients with different vitD status.

Discussion

In this first study investigating 25(OH)D levels in Bulgarian women with PCOS, patients with obesity (with or without PCOS) had lower levels of 25(OH)D compared to lean PCOS subjects, although no statistical significance was found. 25(OH)D levels were independent of PCOS status of the patients included in the study. Attention should be drawn to the fact that approximately 2/3 of the patients in the three groups were VitD-deficient [25(OH)D < 50 nnmol/L].

Some studies [1, 4, 5] show that VitD insufficiency is linked to obesity, insulin resistance, and hyperandrogenemia. Patients in our study without overweight or obesity showed the highest levels of 25(OH)D, as demonstrated in other studies [5, 10]. The obese women population had the lowest 25(OH)D levels. We found that VitD levels correlated with BMI and fat percentage and with the indices of visceral obesity – WC and WSR. Women with and without metabolic syndrome however did not differ significantly in 25(OH)D levels. Negative correlation between 25(OH)D levels and androgens (in this case DHEAS) in our study was observed only in lean patients (BMI < 25 kg/m^2).

Women without vitD insufficiency had the lowest rate of insulin resistance, detected by stimulated IRI levels, compared to the groups with vitD insufficiency and deficiency, and the total group. It is interesting to note that this was not observed when using HOMA for detection of insulin resistance. In our study we did not find a significant correlation between 25(OH) D levels and fasting and stimulated BG or IRI. This corresponds to other studies that also did not find a correlation between 25(OH)D levels and insulin resistance or beta-cell function [11]. We agree with the statement made by other authors that BMI was found to be the most powerful predictor of 25(OH) D concentration, whereas insulin sensitivity was not significant in this regard [12].

Some authors have observed stronger associations of serum 25(OH)D with insulin sensitivity in overweight versus normal weight healthy subjects, suggesting that overweight subjects with hypovitaminosis D may benefit more from vitamin D replacement than normal-weight subjects [13]. This was not observed in our study, likely due to the fact that our study included patients with PCOS or obesity, and not a healthy control population.

There is a debate as to whether visceral obesity is a cause or an effect of vitamin D deficiency [14]. The fact that women with lower intakes of calcium and vitamin D are more likely to exhibit excessive adiposity compared with women with higher intakes [15], that vitD levels do not increase after weight loss [14], and that calcium and/or vitamin D supplementation contributes to a beneficial reduction of visceral adipose tissue [16], suggests that vitamin D insufficiency is a cause rather than an effect of increased adiposity. Visceral obesity, on the other hand, worsens both insulin resistance [17] and the clinical presentation of PCOS [18]. The high prevalence of vitamin D deficiency in our study population raises the question of whether vitD supplementation would be beneficial for targeting the metabolic and reproductive disturbances in these women. Further long-term longitudinal studies are needed to evaluate the effect of vitamin D supplementation in patients with PCOS and/or obesity.

Conclusions

(1) There is a very high prevalence of vitamin D deficiency in Bulgarian women with PCOS and/or obesity. (2) 25(OH)D levels are more dependent on weight than on PCOS-status or insulin resistance in this study population.

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