

Assessment of Vitamin D Levels in Women with Polycystic Ovarian Syndrome

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ABSTRACT

Background: polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence of 6–10% in the general population. PCOS is characterized by the following: ovulatory dysfunction resulting in oligo-amenorrhea and/or anovulation, hyperandrogenism and/or hirsutism and the presence of polycystic ovarian morphology by ultrasound. Vitamin D also plays a physiologic role in reproduction including ovarian follicular development and luteinization via altering anti-Müllerian hormone (AMH), signaling, follicle-stimulating hormone sensitivity and progesterone production in human granulosa cells. **Aim of the Work:** this study aimed to compare levels of vitamin D in women with PCOS and healthy fertile women. **Patient and Methods:** this case control study was conducted in Ain Shams Maternity hospital in outpatient infertility clinics and family planning clinic during the period from March 2017 to October 2017. It was included 40 women diagnosed with PCOS based on Rotterdam criteria and 40 fertile women without PCOS who were recruited from family planning clinic. **Results:** 25(OH) vitamin-D level was significantly lower in PCOS group than in the control group. **Conclusion:** 25(OH) vitamin-D was significantly lower in PCOS group than in the control group, but not sufficient to be a diagnostic tool for PCOS. **Recommendation:** larger studies are still needed to clarify the rule of vitamin D in infertility.

Keywords: polycystic ovary syndrome (PCOS), vitamin D, anti-Müllerian hormone (AMH).

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence of 6–10% in the general population. PCOS is characterized by the following: ovulatory dysfunction resulting in oligo-amenorrhea and/or anovulation, hyper-androgenism and/or hirsutism, and the presence of polycystic ovarian morphology by ultrasound⁽¹⁾. It may also create long-term health risks, such as type 2 diabetes, endometrial cancer and cardiovascular disease as it is associated with anovulation, hyperinsulinemia and central obesity⁽²⁾. Vitamin D has important roles in various parts of the body, especially in the bones. The active form of vitamin D plays an important role in bone metabolism, regulation of calcium-phosphorus equilibrium and cell differentiation and proliferation⁽³⁾. Vitamin D also plays a physiologic role in reproduction including ovarian follicular development and luteinization via altering anti-Müllerian hormone (AMH) signalling, follicle-stimulating hormone sensitivity and progesterone production in human granulosa cells⁽⁴⁾. It also affects glucose homeostasis through several roles. The potential influences of vitamin D on glucose homeostasis included the presence of specific vitamin D receptor (VDR) in pancreatic β -cells and skeletal muscle, the expression of 1- α -hydroxylase enzyme which can catalyze the conversion of 25-hydroxy vitamin D [25(OH)D] to 1,25-dihydroxyvitamin D and the

presence of a vitamin D response element in the human insulin gene promoter⁽⁵⁾. It also increases insulin synthesis and secretion and regulation of steroidogenesis in the human ovarian tissue⁽⁶⁾.

Vitamin D deficiency is quite common in the general population. In fact, in several studies, vitamin D levels were found to be below 20 ng/ml in 10-60% of the adults⁽⁷⁾.

Serum 25-hydroxy vitamin D (25-OH D) concentrations below 20 ng/ml were considered as vitamin D deficiency and serum 25-OH D concentrations of 20-30 ng/ml were considered as vitamin D insufficiency⁽⁸⁾. There are many causes of vitamin D deficiency, including reduced skin synthesis and absorption of vitamin D and acquired and heritable disorders of vitamin D metabolism and responsiveness. Use of sunscreen, presence of pigment in the skin, patients with skin grafts for burns will cause a reduction in the synthesis of vitamin D. Obesity can cause decreased bioavailability due to reduced availability of vitamin D. The presence of liver failure and kidney disease can also affect the metabolism of vitamin D⁽⁸⁾. The underlying pathogenesis of PCOS included insulin resistance and compensatory hyperinsulinemia⁽⁹⁾. Increased insulin resistance causes an increase in weight which triggers hyperandrogenism and, thus, results in clinical symptoms. Although insulin resistance more frequently appears in obese patients (65%), it is less frequent in lean patients with PCOS

(20%)⁽¹⁰⁾. A study compared vitamin D levels between patients with PCOS and healthy women with normal ovulation ; there was no change in vitamin D levels in patients with PCOS as compared to the control ones⁽¹⁾. Others studies showed higher levels⁽¹¹⁾ or low levels of vitamin D⁽¹²⁾. It has been found that body mass index (BMI) and insulin resistance were negatively correlated with vitamin D levels and obese patients have been reported to have low levels of vitamin D⁽¹²⁾. One study revealed that insulin resistance was not correlated with vitamin D and that obesity was associated with low vitamin D levels⁽¹³⁾. Study of **Palm *et al.***⁽¹⁴⁾ on obese patients with PCOS showed that insulin resistance parameters did not change after three-month supplementation of vitamin D, while total testosterone and androstenedion levels were decreased. In an observational study on 25 women with PCOS and 27 controls, **Li *et al.*** reported an inverse correlation between the 25(OH)D levels and BMI, 72% of women with PCOS had vitamin D deficiency, of which 44% with severe deficiency⁽¹⁵⁾. The same association was observed by **Wehr *et al.***⁽¹⁶⁾ in an observational study involved 206 PCOS women with 72% of the population showed the evidence of vitamin D deficiency (25(OH)D serum < 30 ng/ml) and noted the existence of an inverse association between 25(OH)D levels and BMI.

AIM OF THE WORK

Objective of this study was to compare levels of vitamin D in women with PCOS and healthy fertile women.

PATIENTS AND METHODS

The study was approved by the Ethics Board of Ain Shams University.

1-Study type:

This was a case control study.

2- Study setting:

The current study was held at Ain Shams Maternity hospital in outpatient infertility clinics and family planning clinic.

3- Study timing:

March 2017 to October 2017

4-Study population:

1- Group A: 40 women diagnosed with PCOS based on **Rotterdam** criteria⁽¹⁷⁾. These criteria included 2 of 3: different clinical manifestations of oligo-ovulation or hyperandrogenism (clinical or laboratory), including hirsutism, acne, elevated testosterone, as well as morphologic view of PCOS in ovarian sonography.

2- Group B: 40 fertile women without PCOS who were recruited from family planning clinic.

Sample size justification:

Sample size was calculated using PASS[®] version 11 program, setting the type-1 error (α) at 0.05 (95% Confidence interval) and the power (1- β) at 0.8. Results from a previous study⁽¹⁸⁾ showed that vitamin D level among PCO was 25.5 ± 7.1 ng, while among controls the vitamin d levels was 35.3 ± 8.1 ng. Calculation according to these values produced a minimal sample size of 40 cases per group.

Inclusion criteria:

- 1- Women aged 18-35 years.
- 2- Women who had been diagnosed with PCOS based on Rotterdam criteria . This criteria included 2 of 3:
 - a) Different clinical manifestations of oligo-ovulation
 - b) Hyperandrogenism (clinical or laboratory), including hirsutism, acne, elevated testosterone.
 - c) Morphologic view of PCOS in ovarian sonography. Meaning presence of 12 or more follicles measuring 2-9 mm in diameter in each ovary and/or ovarian volume more than 10 cm³.
- 3- Matched control group for age and BMI are women without PCOS defined as healthy fertile women in reproductive age with regular cycles who were coming to family planning clinic for routine checkup or IUCD insertion.

Exclusion criteria

- 1- Women with any endocrine disorders (thyroid, parathyroid, diabetes mellitus, Cushing syndrome, congenital adrenal hyperplasia, hyperprolactinemia).
- 2- Women with renal, liver or chronic diseases.
- 3- Women who used medications suspicious to affect vitamin D concentrations during 6 months prior to the study.
- 4- Women who had a history of smoking or drug abuse.
- 5- Women who had a history of hormonal treatment in the last 3 months prior to the study.

METHODS

1- Verbal consent was taken from each patient after explanation of the procedure completely.

2- All patients were subjected to:

a- History taking

- Personal history (name-age-resident-occupation-special habits- husband history-menstrual history).

- Past history included medical (diseases-drugs) and surgical history (previous operation).

b- Clinical examination: General examination included BMI = kg/m².

c- Investigations: Serum vitamin D level.

INTERVENTION

All women with PCOS and without PCOS were subjected to:

- 1- Weight was measured in light indoor clothing and barefoot and height was measured in stand up position. Then, the following formula was used to calculate the body mass index: $BMI = \text{kg}/\text{m}^2$. Based on BMI findings, the participants were divided into four following groups: less than 18,5 kg/m^2 as underweight, 18,5-22,9 kg/m^2 as norm weight, 23-24,9 kg/m^2 as overweight and 25 kg/m^2 or more as obese ⁽¹⁸⁾.
- 2- Serum concentration of 25(OH) D was measured by Elisa technique.

Technique of measurement of serum vitamin D level:

Specimen Collection and Handling

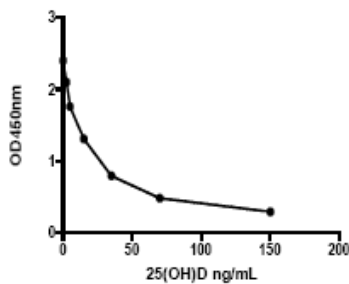
- Whole blood specimen were collected by puncture of anticubital vien then 2ml blood was withdrawn by usual plastic syringe then blood sample was kept in plain test tube. Separation of the serum by centrifugation at a speed of 4000 rpm was done. Then all serum samples were refrigerated at -20c up to whole specimens was

collected for ELISA technique (Cal biotech 25(OH) Vitamin D ELISA kits) on Starfax 2100 device.

- Heamolized sample was rejected.



Figure 1: Starfax 2100 device



25(OH)D, (ng/ml)	Absorbance (450nm)
0	2.40
2.5	2.10
5	1.76
15	1.31
35	0.79
70	0.48
150	0.29

Figure 2: STANDARD CURVE

RESULTS

Results were expressed in ng/mL.

Table 1: reference range according to calbiotech25 (OH)vitamin d ELISA kits:

Deficient : 10ng/ml
Insufficient : 10ng/ml-30ng/ml
Sufficient : 30ng/ml-100ng/ml
Intoxication: 100ng/ml

Table 2: comparison between the studied groups regarding age and BMI

Findings	Measures	PCOS (N=40)	Control (N=40)	P
Age (years)	Mean±SD	27.0±3.9	27.4±3.6	^
	Range	19.0–35.0	21.0–34.0	0.593
BMI (kg/m^2)	Mean±SD	27.2±3.8	27.6±4.0	^
	Range	16.9–33.0	18.3–36.4	0.716
BMI grades	Underweight	2 (5.0%)	1 (2.5%)	# 0.895
	Normal	9 (22.5%)	7 (17.5%)	
	Overweight	21 (52.5%)	23 (57.5%)	
	Obese	8 (20.0%)	9 (22.5%)	

^Independent t-test, #Fisher's Exact test, *Significant

Table 2 showed that there was no significant statistical difference between the studied groups regarding age and BMI.

Table 3: comparison between the studied groups regarding 25(OH) vitamin-D level.

Findings	Measures	PCOS (N=40)	Control (N=40)	P
Level (ng/mL)	Mean±SD	7.7±3.9	12.7±8.6	^ <0.001*
	Range	2.0–15.0	3.0–40.0	
Grades	Normal	0 (0.0%)	3 (7.5%)	# 0.043*
	Deficiency	13 (32.5%)	19 (47.5%)	
	Insufficiency	27 (67.5%)	18 (45.0%)	

^Independent t-test, #fisher's Exact test, *Significant

Table 3 showed that 25(OH) vitamin-D level was significantly lower in PCOS group than in the control group.

Table 4: correlation between 25(OH) vitamin-D and age and BMI.

Variables	PCOS (N=40)		Control (N=40)	
	R	P	R	p
Age	0.166	0.305	0.224	0.166
BMI	0.138	0.397	0.038	0.815

Pearson correlation

Table 4 showed that there was no significant statistical correlations between 25(OH) vitamin-D and age& BMI.

Table 5: diagnostic performance of age, BMI and 25(OH) vitamin-D in diagnosing PCOS

Factors	AUC	SE	P	95% CI	Cut off
25(OH) vitamin-D	0.676	0.059	0.007*	0.560–0.792	≤11.0

AUC. Area under curve, SE. standard error, CI. Confidence interval, *significant

Table 5 showed that: 25(OH) vitamin-D had significant weak diagnostic performance in diagnosing PCOS.

Table 6: diagnostic characteristics of 25(OH) vitamin-D ≤11.0 (ng/mL) in diagnosing PCOS

Characters	Value	95% CI
Sensitivity	82.5%	67.2%–92.7%
Specificity	40.0%	24.9%–56.7%
Diagnostic accuracy (DA)	61.3%	49.7%–71.9%
Youden's index	22.5%	3.3%–41.7%
Positive Predictive value (PPV)	57.9%	44.1%–70.9%
Negative Predictive value (NPV)	69.6%	47.1%–86.8%
Positive likelihood ratio (LR+)	1.38	1.028–1.839
Negative likelihood ratio (LR-)	2.29	1.056–4.949
Diagnostic odd ratio (LR)	3.14	1.120–8.822
Kappa	0.23	0.032–0.418

CI: Confidence interval

Table 6 showed that 25(OH) vitamin-D level ≤11.0 (ng/mL) had moderate sensitivity and weak other diagnostic characteristics in diagnosing PCOS.

DISCUSSION

In this case-control study levels of vitamin D in women with PCOS and healthy fertile women were compared. The results indicated that 25(OH) vitamin-D was significantly lower in PCOS group

than in control group, but not sufficient to be a diagnostic tool for PCOS. And There were no significant correlations between 25(OH) vitamin-D and age and BMI.

In agreement with the current study a study conducted by **Elida *et al.*** ⁽¹⁸⁾ found that vitamin D levels are higher in the control group (women without PCOS) than women with PCOS. Independent t-test indicated a significant association between the incidence of PCOS and vitamin D levels statistically (p value <0.05). In this study a total of 46 participants were assessed based on age and body mass index. Vitamin D levels decreased with increased body mass index. The lowest vitamin D level was in obese women. This difference was statistically significant at ($p <0.05$) in contrast to the current study where there was no association with BMI. Results of a study carried out by **Mazloomi *et al.*** ⁽¹⁹⁾ showed low levels of vitamin D independent of BMI and insulin resistance in the patients with PCOS than the control in a study of 103 women with PCOS and another 103 control women, the same as results of the current study. Previous study conducted by **Li *et al.*** ⁽¹⁾ showed an observational study on 25 women with PCOS and 27 controls, an inverse correlation between the 25(OH)D levels and BMI and 72% of women with PCOS had vitamin D deficiency, of which 44% with severe deficiency. **Wehr *et al.*** ⁽¹⁶⁾ reported also lower serum vitamin D levels in a large number of women with PCOS ($n=545$) compared to the controls ($n=145$) (25.7 vs. 32.0 ng/mL, respectively), in concordance with the current study, but they found an inverse association between 25(OH) D and BMI in contrast with the current study.

Kumar *et al.* ⁽²⁰⁾ held a study comprised of 80 newly diagnosed polycystic ovary syndrome women in the age range of 23 to 33 years. The biochemical parameters measured in the study included fasting blood sugar, fasting insulin, calcium, phosphorus and vitamin D. The values obtained were compared with equal number of age and body mass indexed (BMI) matched healthy controls. Levels of 25 – OH vitamin D were decreased in PCOS women when compared to the healthy controls and there was no significant statistical difference with respect age and BMI was the same as with the current study.

Roshan and Iroshan ⁽²¹⁾ studied a total of 314 cases of infertility. 157 infertile women with PCOS as case and another 157 infertile women as the control group. The mean serum vitamin D concentration in case group was significantly lower than in the control group (13 ± 11.5 ng/ml and 25.07 ± 2.07 ng/ml, respectively) ($P < 0.001$). Vitamin D deficiency in infertile PCOS women was more prevalent than the other infertile

patients. Therefore, measuring serum vitamin D especially in infertile PCOS was suggested.

Setenay *et al.* ⁽²²⁾ studied a forty eight normal-weight (body mass index (BMI) of 19-24.99 kg/m²) women with PCOS, 36 overweight (BMI of 25-29.9 kg/m²) women with PCOS and 56 normal-weight controls participated in the study. Blood samples were collected in the early follicular phase (between day 2 and day 5 of the menstrual cycle) at 9:00 am after an overnight fast. Circulating concentrations of 25-OH D, luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, TSH, free testosterone, dehydroepiandrosterone sulphate (DHEA-SO₄), 17-hydroxyprogesterone, sex hormone binding globulin (SHBG), fasting insulin, fasting glucose, and lipid profile were assessed. Their results showed that normal weight (BMI 19-24.99 kg/m²) and overweight (BMI 25-29.99 kg/m²) women with PCOS were compared to normal-weight controls and lower 25-OH D levels were found in both PCOS groups ($p < 0.05$). In contrast with the current study **Kim *et al.*** ⁽²³⁾, found no significant statistical differences between the 2 groups (PCOS patients and the control group) as regarding serum vitamin D level and clinical or metabolic profiles in both, they 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.

Güdücü *et al.* ⁽²⁴⁾ also found that there was no difference in 25-hydroxyvitamin D levels of women with PCOS and the control group. But, they compared biochemical, hormonal parameters (insulin resistance) and 25 hydroxyvitamin D levels of 58 women with PCOS and 38 body mass index matched controls. Low 25-hydroxyvitamin D levels in women with PCOS were related to higher insulin levels. Although, the negative relationship between 25- hydroxyvitamin D levels and body mass index did not reach statistical significance the same with the current study.

Ghadimi *et al.* ⁽²⁵⁾ study concluded that as all PCOs subjects had hypovitaminosis D, they compared the differences of some variables like signs of PCOs in two subgroups including vitamin D insufficiency (10-30 ng/ml) and deficiency (<10 ng/ml) in 104 high school girls(case group) with PCOs and 88 healthy girls(case group). Serum samples were used for determination of 25(OH) D, calcium, fasting plasma glucose (FPG) and insulin (FPI), total testosterone, free testosterone, dehydroepiandrosterone sulfate

(DHEAS), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total cholesterol, HDL and LDL cholesterol and triglycerides in the two groups. There was no relationship with the severity of hypovitaminosis D and the presence of acne, hirsutism, polycystic ovaries and central obesity. Based on Pearson's test, no significant correlations were found between 25(OH) D levels with lipid profile, FBS, fasting insulin and endocrine parameters such as testosterone, free testosterone, FSH, LH, TSH and prolactin.

In a study carried out by **Panadis *et al.*** ⁽²⁶⁾, there was no difference in vitamin D levels between healthy individuals and patients with PCOS in general, but obese patients with PCOS had lower vitamin D levels.

The studies carried out by **Ngo *et al.*** ⁽²⁷⁾ and **Mahmoudi *et al.*** ⁽²⁸⁾ found a higher serum 25OH D level in PCOS women than in the control women

In a study carried out by **Moini *et al.*** ⁽²⁹⁾ a cross sectional study was performed on 242 women, 125 patients with PCOS and 117 healthy individuals resulted in a high incidence of 25(OH)D deficiency in both PCOS and normal groups. Although the PCOS group had lower levels of calcium and 25(OH) D, but the difference was not considerable and the level of 25(OH) D did not differ significantly between the two sub-groups (obese and non-obese)

In a study carried out by **Figurová *et al.*** ⁽³⁰⁾ on 99 PCOS women and 66 controls. Their result reported that there was no significant difference in 25(OH) D levels between PCOS women and the controls (24.79 ± 10.77 vs 25.07 ± 10.14 ng/ml, $p = 0.868$) and also in the prevalence of 25(OH)D deficiency in both groups (80 vs 70 %; $p = 0.138$). Vitamin D-deficient PCOS patients had significantly higher body mass index (BMI) and fasting insulin, compared to vitamin D-deficient controls. PCOS women with metabolic syndrome (MS) had lower serum 25(OH)D compared to those without MS (20.6 ± 8.3 vs 25.9 ± 11.3 ng/ml, $p = 0.049$). A study carried out by **Sadhir *et al.*** ⁽³¹⁾ on 107 participants. 37 of the included participants were in the PCOS group and 70 were in the control group, with a mean age of 15.2 years. In the PCOS group, 97.2% were obese and vitamin D deficiency was noted among 62.2% females. The mean serum 25(OH)D level was 18.4 and 21.6 ng/mL in PCOS and the control group, respectively. The difference in mean 25(OH)D levels between the 2 groups was not statistically

significant ($P > .05$) when controlled for ethnicity, body mass index percentile, and season.

CONCLUSION AND RECOMMENDATIONS

25(OH) Vitamin-D was significantly lower in PCOS group than in the control group, but not sufficient to be a diagnostic tool for PCOS.

Larger studies are still needed to clarify the rule of vitamin D in infertility.

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