Vitamin D status in polycystic ovary syndrome

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Background
Vitamin D deficiency (VDD) is an important public health problem worldwide, and polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age, with prevalence up to 10%. It is characterized by ovulatory dysfunction, resulting in oligomenorrhea and/or anovulation, hyperandrogenism, and polycystic ovarian morphology by ultrasound. Metabolic disturbances are present in most women with PCOS, including impaired glucose tolerance and insulin resistance (IR) with compensatory hyperinsulinemia. It may also create health risks such as T2DM, endometrial cancer, and cardiovascular disease. Accumulating evidence from several studies suggests that VDD may be involved in the pathogenesis of PCOS as the possible missing link between IR and PCOS. The aim of this study was to evaluate the suggested role of vitamin D in PCOS.

Participants and methods
The study included 70 women in reproductive age (16–44 years old) divided into two groups: group I included 50 women in reproductive age with PCOS, and group II included 20 healthy women in reproductive age with regular menstrual cycles. All were subjected to history taking; clinical examination, including blood pressure measurement; anthropometric measurements, such as body weight, height, and calculation of BMI, and waist and hip circumference with calculation of the waist/hip ratio; skin examination for acanthosis nigricans (sign of IR) and signs of androgen excess, such as hirsutism, androgenic alopecia, and acne; laboratory investigations, such as fasting blood glucose, lipid profile (total cholesterol, serum triglycerides, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol), serum levels of ionized calcium, serum levels of 25 (OH) vitamin D3, serum insulin level with calculation of Homeostatic Model Assessment of Insulin Resistance, serum luteinizing hormone, serum follicle-stimulating hormone with calculation of luteinizing hormone/follicle-stimulating hormone ratio, serum prolactin, serum total testosterone, and sex hormone-binding globulin with calculation of free androgen index; and imaging studies, such as pelvic ultrasonography with a 3.5 MHz convex electronic probe to examine the ovaries or transvaginal ultrasound.

Results
Serum 25 OH vitamin D level was statistically significantly lower in group I (women with PCOS) than group II (the control group) (mean: 6.05±2.56 vs 21.58±1.92 ng/ml) ($P< 0.001$). There was a statistically significant positive correlation between serum 25 (OH) vitamin D level and serum ionized calcium (r=0.465, P=0.001) and sex hormone-binding globulin (r=0.407, P=0.003). However, there was a statistically significant negative correlation between serum 25 (OH) vitamin D level and BMI (r=–0.363, P=0.010), waist/hip ratio (r=–0.255, P=0.049), serum fasting insulin level (r=-0.487, $P<0.001$), Homeostatic Model Assessment of Insulin Resistance (r=–0.521, $P<0.001$), serum total testosterone (r=–0.418, P=0.003), free androgen index (r=–0.597, $P<0.001$), right ovarian volume (r=–0.44, P=0.001), left ovarian volume(r=-0.407, P=0.003), total ovarian volume (r=–0.447, P=0.001), right ovarian follicular number (r=–0.445, P=0.001), left ovarian follicular number (r=–0.488, $P< 0.001$), and total ovarian follicular number (r=–0.474, P=0.001).

Conclusion
VDD is very common in women with PCOS and is associated with metabolic derangement, including IR, cardiovascular risk factors, as well as ovulatory dysfunction, infertility, and hirsutism.

Keywords:
hyperandrogenism, insulin resistance (IR), ovulatory dysfunction, polycystic ovary syndrome (PCOS), vitamin D deficiency (VDD)

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Introduction

Vitamin D deficiency (VDD) is an important public health problem worldwide. VDD may be associated with its well-known calcemic effect as well as a broad spectrum of pleiotropic effects. Hence, the problem of VDD and its adequate supply represent an important issue in public health and clinical practice [1–3].

Polycystic ovary syndrome (PCOS) is a significant public health issue with reproductive, metabolic, and psychological features. It is one of the most common conditions in reproductive aged women, affecting 8–13%, with up to 70% remaining undiagnosed. It is characterized by ovulatory dysfunction resulting in oligomenorrhea and/or anovulation, hyperandrogenism, and polycystic ovarian morphology by ultrasound. Metabolic disturbances are present in most women with PCOS, including impaired glucose tolerance and insulin resistance (IR) with compensatory hyperinsulinemia. It may also create health risks such as T2DM, endometrial cancer, and cardiovascular disease, being associated with anovulation, hyperinsulinemia, and central obesity. Adipose tissue dysfunction acts as a contributor to IR in PCOS. However, a substantial number of lean women with PCOS have IR independent of obesity [4–15].

Accumulating evidences from several studies suggest that VDD may be involved in several features of PCOS, such as infertility, hirsutism, IR, and cardiovascular risk. It has been proposed as the possible missing link between IR and PCOS [1–3,16–18].

In this study, we aimed to evaluate the suggested role of vitamin D in PCOS to improve screening and therapy in women with PCOS.

Participants and methods

The study included 70 women in reproductive age (16–44 years old) divided into two groups: group I had 50 women in reproductive age with PCOS attending Endocrinology and Gynecology clinic at Alexandria University Hospitals, and group II had 20 healthy women defined as women in reproductive age with regular menstrual cycles.

PCOS diagnosis was based on International evidence-based guideline for the assessment and management of PCOS 2018 and the revised Rotterdam consensus criteria. In the revised Rotterdam criteria, 2 of 3 criteria are required for diagnosis: oligo and/or anovulation, clinical (as hirsutism and/or acne) and/or biochemical (elevated androgens) signs of hyperandrogenism, and polycystic ovaries in ultrasound. Oligo or anovulation is defined as cycles less than 21 days or more than 35 days. Polycystic ovarian morphology is defined as greater than or equal to 12 follicles 2–9 mm in diameter [4,19–23].

Exclusion criteria included current pregnancy or breast feeding women; women with conditions known to affect vitamin D level, either metabolism like renal disease and liver disease or absorption such as gastrointestinal problems, for example, inflammatory bowel diseases, calcium and vitamin D supplementation during 6 months before the study; women with conditions or tumors known to affect the androgen levels, for example, congenital adrenal hyperplasia, hyperprolactinemia, current or previous (within the last 6 months) use of androgen preparations, oral contraceptives, glucocorticoids, antiandrogens, ovulation induction agents, or other hormonal drugs known to affect the plasma sex steroid level; women with Cushing's syndrome; women with thyroid dysfunction; and women who smoked [24,25].

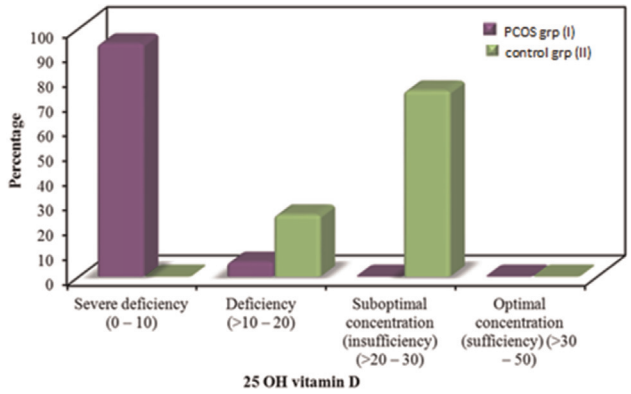
All selected women were subjected to history taking; clinical examination including blood pressure measurement; anthropometric measurements, such as accurate measurement of body weight, height, and calculation of BMI, as well as waist and hip circumference measurements with calculation of the waist/hip ratio (WHR); and skin examination for acanthosis nigricans (sign of IR) and signs of androgen excess such as hirsutism, androgenic alopecia, and acne. The severity of hirsutism was assessed by modified Ferriman-Gallwey scoring system. Laboratory investigations included the following: regularly menstruating women were scanned in the early follicular phase (days 3–5), and oligo-/amenorrhoeic women were scanned at random. Venous blood samples were withdrawn from every participant after an overnight fast (12 h) for examination of fasting blood glucose; lipid profile including total cholesterol, serum triglycerides, low-

Table 1 Comparison between the two studied groups according to 25 OH vitamin D

25 OH vitamin D (ng/ml)	PCOS group (group I) (n=50) [n (%)]	Control group (group II) (n=20) [n (%)]	Test of significance	P
Severe deficiency (0–10)	47 (94.0)	0	χ^2=66.314*	^{MC}P<0.001*
Deficiency (>10–20)	3 (6.0)	5 (25.0)		
Suboptimal concentration (insufficiency) (>20–30)	0	15 (75.0)		
Optimal concentration (sufficiency) (>30–50)	0	0		
Minimum–maximum	3.0–14.90	18.40–24.60	U=0.0*	<0.001*
Mean±SD	6.05±2.56	21.58±1.92		
Median (IQR)	5.40 (4.1–7.8)	21.95 (19.7–22.9)		

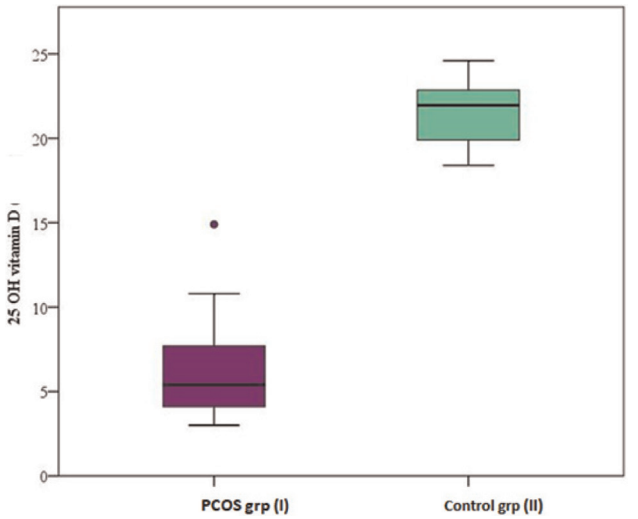
MC, Monte Carlo; P, P value for comparing between the studied groups; PCOS, polycystic ovary syndrome; U, Mann–Whitney test.
*Statistically significant at $P \leq 0.05$.

Figure 1



Comparison between the two studied groups according to 25 OH vitamin D in ng/ml.

Figure 2



Comparison between the two studied groups according to 25 OH vitamin D in ng/ml.

density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol; serum levels of ionized calcium; serum levels of 25 (OH) vitamin D by electrochemiluminescence; and hormonal assay by electrochemiluminescence for serum insulin level and the calculation of Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), serum luteinizing hormone, serum follicle-stimulating hormone with calculation of luteinizing hormone/follicle-stimulating hormone ratio, serum prolactin, serum total testosterone, sex hormone-binding globulin (SHBG), and the calculation of free androgen index (FAI). Imaging studies included pelvic ultrasonography with a 3.5 MHz convex electronic probe to examine the ovaries or transvaginal ultrasound.

Results

In this study, it was found that in the PCOS group, 47/50 women with PCOS (94%) had shown severe VDD (serum 25 OH vitamin D level ranged from 0 to 10 ng/ml) and 3/50 (6%) had shown VDD (serum 25 OH vitamin D level ranged from 10 to 20 ng/ml). In group I, serum 25 OH vitamin D ranged from 3 to 14.9 ng/ml, with a mean of 6.05±2.56 ng/ml. However, in the control group, 15/20 women in the control group (75%) had shown vitamin D suboptimal concentration (insufficiency) (serum 25 OH vitamin D level ranged from 20 to 30 ng/ml. However, 5/20 women (25%) had shown deficiency (serum 25 OH vitamin D ranged from 10 to 20 ng/ml). In group II, serum 25 OH vitamin D ranged from 18.4 to 24.6 ng/ml, with a mean of 21.58±1.92 ng/ml. Serum 25 OH vitamin D level was statistically significantly lower in group I (women with PCOS) than group II (the control group) (mean: 6.05±2.56 vs 21.58±1.92 ng/ml) (P<0.001) (Table 1, Figs 1 and 2).

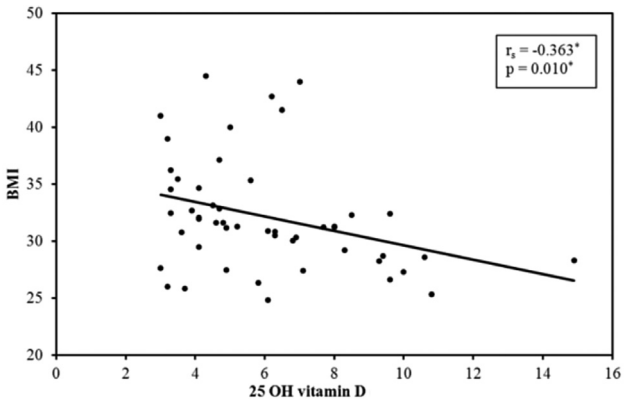
There was a statistically significant positive correlation between serum 25 (OH) vitamin D level and serum ionized calcium (r=0.465, P=0.001) and SHBG (r=0.407, P=0.003). However, there was a statistically significant negative correlation between serum 25 (OH) vitamin D level and BMI (r=–0.363, P=0.010), WHR (r=–0.255, P=0.049), serum fasting insulin level (r=-0.487, P<0.001), HOMA- IR

Table 2 Correlation between 25 OH vitamin D and different parameters in the polycystic ovary syndrome group (*n*=50)

	25 OH vitamin D (mg/dl)	
	r_s	*P*
BMI	–0.363	0.010*
Waist-to-hip circumference ratio	–0.255*	0.049*
Systolic blood pressure	0.052	0.721
Diastolic blood pressure	0.138	0.341
FBG	–0.216	0.131
TCH (mg/dl)	-0.194	0.178
TG (mg/dl)	-0.262	0.066
HDL (mg/dl)	-0.052	0.718
LDL (mg/dl)	-0.261	0.067
Serum ionized calcium (mg/dl)	0.465*	0.001*
Fasting insulin (IU/ml)	-0.487*	<0.001*
HOMA-IR	-0.521*	<0.001*
LH (U/l)	0.007	0.962
FSH (U/l)	0.052	0.721
LH/FSH	-0.025	0.863
T. testosterone (nmol/l)	-0.418*	0.003*
Sex hormone-binding globulin (nmol/l)	0.407*	0.003*
Free androgen index	-0.597*	<0.001*
Right ovarian volume (cm^3)	–0.440*	0.001*
Left ovarian volume (cm^3)	–0.407*	0.003*
Total ovarian volume (cm^3)	–0.447*	0.001*
Right ovarian follicular number	–0.445*	0.001*
Left ovarian follicular number	–0.488*	<0.001*
Total ovarian follicular number	–0.474*	0.001*

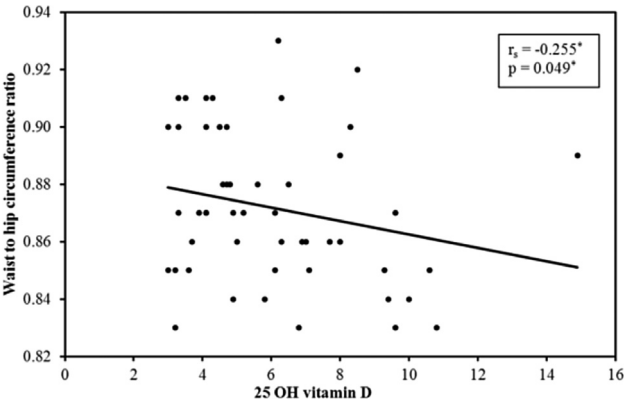
FBG, fasting blood glucose; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; LDL, low-density lipoprotein; LH, luteinizing hormone; r_s, Spearman coefficient; TCH, total cholesterol; TG, triglyceride. *Statistically significant at $P \leq 0.05$.

Figure 3



Correlation between serum 25 OH vitamin D and BMI in the polycystic ovary syndrome group.

Figure 4



Correlation between serum 25 OH vitamin D and waist-to-hip circumference ratio in the polycystic ovary syndrome group.

(*r*=–0.521, *P*<0.001), serum total testosterone (*r*=–0.418, *P*=0.003), FAI (*r*=–0.597, *P*<0.001), right ovarian volume (*r*=–0.44, *P*=0.001), left ovarian volume(r=-0.407, *P*=0.003), total ovarian volume (*r*=–0.447, *P*=0.001), right ovarian follicular number (*r*=–0.445, *P*=0.001), left ovarian follicular number (*r*=–0.488, *P*< 0.001), and total ovarian follicular number (*r*=–0.474, *P*=0.001) (Table 2, Figs 3–12).

Discussion

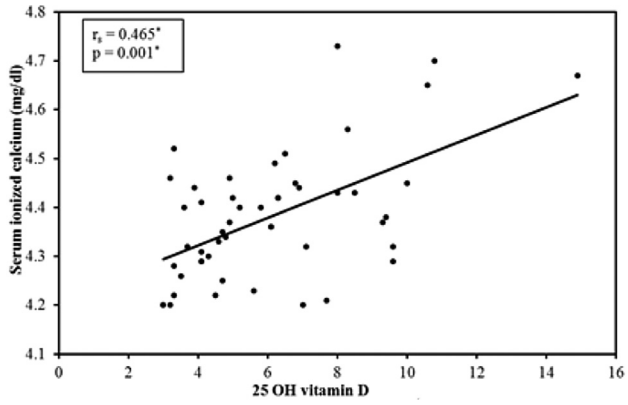
Accumulating evidence from several studies suggest that VDD may be involved in the pathogenesis of PCOS [1–3,16–18].

The aim of this study was to evaluate the suggested role of vitamin D in PCOS. The study included 70 women in reproductive age divided into two groups: group I had 50 women with PCOS and group II had 20 healthy women with regular menstrual cycles.

In the current study, it was found that, in group I, 47/50 women with PCOS (94%) had shown severe VDD (serum 25 OH vitamin D level ranged from 0 to 10 ng/ml) and 3/50 (6%) had shown VDD (serum 25 OH vitamin D level ranged from 10 to 20 ng/ml). However, in group II, 15/20 women in the control group (75%) had shown vitamin D suboptimal concentration (insufficiency) (serum 25 OH vitamin D level ranged from 20 to 30 ng/ml, whereas 5/20 women (25%) had shown deficiency (serum 25 OH vitamin D ranged from 10to– 20 ng/ml). Serum 25 OH vitamin D level was statistically significantly lower in group I (women with PCOS) than group II (the control group) (mean: 6.05±2.56 vs 21.58±1.92 ng/ml) (*P*<0.001).

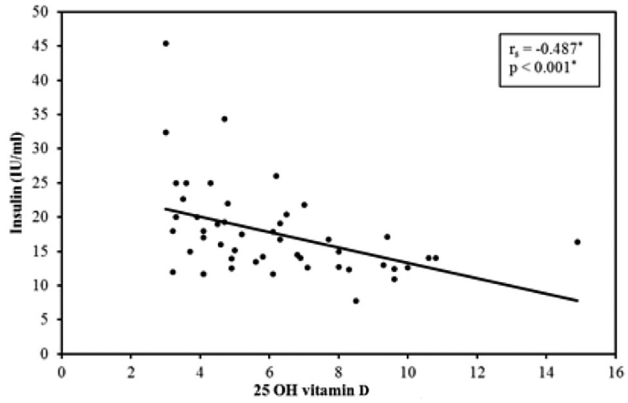
Supporting our work, a cross-sectional study conducted by Krul-Poel and colleagues that compared vitamin D status between 639 women with PCOS and 449 fertile women showed that serum 25(OH)D was significantly lower in women with PCOS compared with controls [mean 25(OH)D of 49.0 vs 64.5 nmol/l]. In the systemic review published in the European Journal

Figure 5



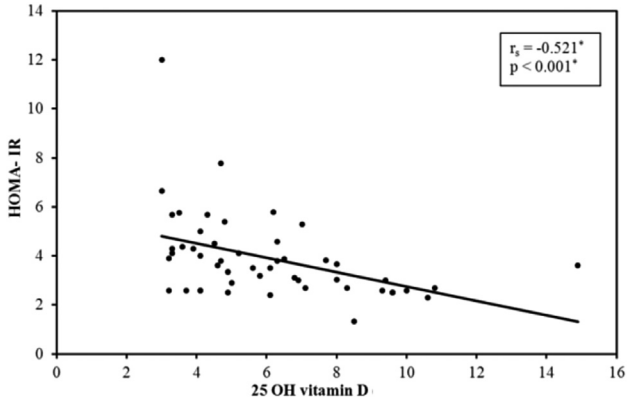
Correlation between serum 25 OH vitamin D and serum ionized calcium(mg/dl) in the polycystic ovary syndrome group.

Figure 6



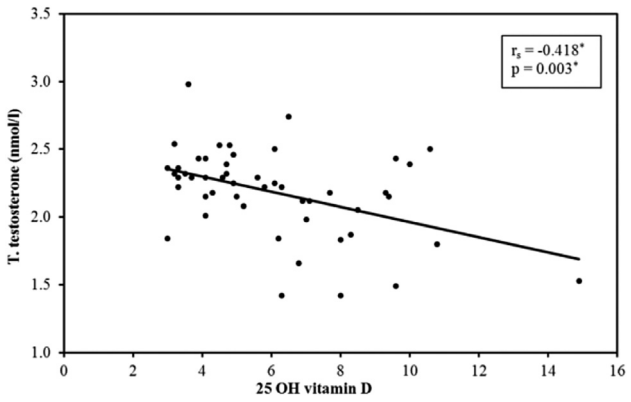
Correlation between serum 25 OH vitamin D and serum insulin (IU/ml) in the polycystic ovary syndrome group.

Figure 7



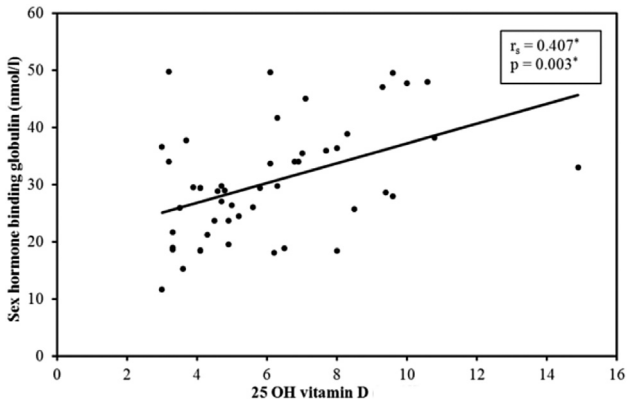
Correlation between serum 25 OH vitamin D and Homeostatic Model Assessment of Insulin Resistance in the polycystic ovary syndrome group.

Figure 8



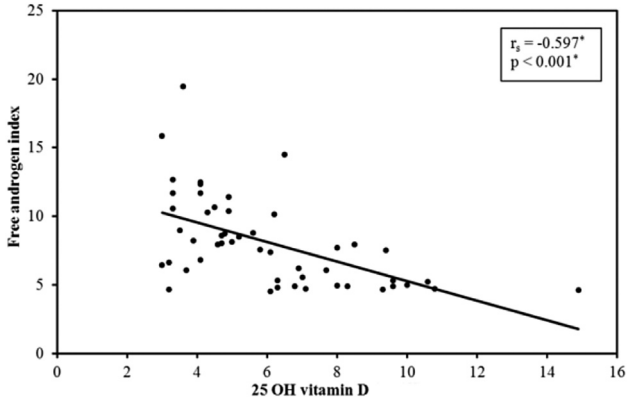
Correlation between serum 25 OH vitamin D and T. testosterone in the polycystic ovary syndrome group.

Figure 9



Correlation between serum 25 OH vitamin D and sex hormone-binding globulin (nmol/l) in the polycystic ovary syndrome group.

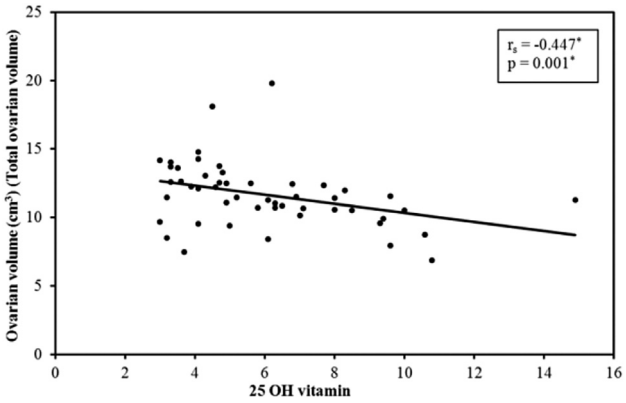
Figure 10



Correlation between serum 25 OH vitamin D and free androgen index in the polycystic ovary syndrome group.

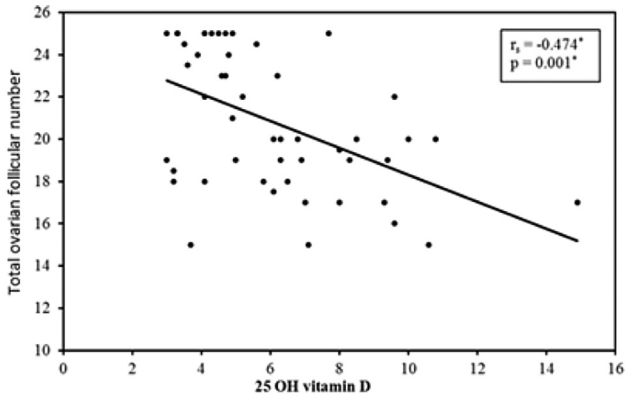
of Endocrinology (2013) by Krul-Poel and colleagues, three studies demonstrated a significantly lower serum 25OHD level in women with PCOS: 32.4 vs 73.7 nmol/l in 90 PCOS women and 47 control women by Savastano and colleagues, 30.0 vs 43.7 nmol/l among 103 women with PCOS and their controls by Mazloomi and colleagues, and 17.7 vs 79.2 nmol/l in 30 women with PCOS and 15 control women by Hassan and colleagues [17,18,26–28].

Figure 11



Correlation between serum 25 OH vitamin D and total ovarian volume in the polycystic ovary syndrome group.

Figure 12



Correlation between serum 25 OH vitamin D and total ovarian follicular number in the polycystic ovary syndrome group.

Thomson and colleagues in a review article in *Clinical Endocrinology Journal* (2012) stated that several studies have reported low levels of vitamin D in women with PCOS with the majority having values less than 20 ng/ml (67–85%). Wehr and colleagues agreed and reported lower levels in women with PCOS (n=545) compared with the control women (n=145; 25·7 vs 32·0 ng/ml, respectively). On the contrary, Li and colleagues reported lower vitamin D levels, although not significant, in women with PCOS compared with women without PCOS (11 ng/ml in PCOS group vs 17 ng/ml in control group) [29–31].

In contrary to our results, Moini and colleagues compared 25(OH)D level between 117 normal and 125 PCOS cases in Arash Hospital, Tehran, Iran. Their results showed no significant differences between both groups (P=0.65). They concluded that the high prevalence of VDD may have influenced their results. Another study in South Indian population by Lakshman and colleagues concluded that the majority of patients with PCOS and controls had VDD, and there was no significant difference in PCOS group and controls. The mean vitamin D level in the PCOS group was even higher in the PCOS group (15.45±7.88 vs 12.83±5.76 ng/ml in the control group). The researchers provided explanation that the controls were hospital workers and were not exposed adequately to sunlight whereas patients in the PCOS group were unemployed women and students who were exposed to more sunlight. Moreover, Kim and colleagues in Seoul Korea found no differences between patients with PCOS and controls (19.6±6.6 vs 20.1±7.4 ng/ml respectively, P=0.696) and stated that VDD is a common finding among patients with PCOS and controls [24,25,32].

In the current study results, there was a statistically significant positive correlation between serum 25 (OH) vitamin D level and serum ionized calcium (r=0.465, P=0.001) and SHBG (r=0.407, P=0.003). However, there was a statistically significant negative correlation between serum 25 (OH) vitamin D level and BMI (r=–0.363, P=0.010), WHR (r=–0.255, P=0.049), serum fasting insulin level (r=–0.487, P<0.001), HOMA- IR (r=–0.521, P<0.001), serum total testosterone (r=–0.418, P=0.003), FAI (r=–0.597, P<0.001), right ovarian volume (r=–0.44, P=0.001), left ovarian volume(r=-0.407, P=0.003), total ovarian volume (r=–0.447, P=0.001), right ovarian follicular number (r=–0.445, P=0.001), left ovarian follicular number (r=–0.488, P<0.001), and total ovarian follicular number (r=–0.474, P=0.001).

In agreement with our results, the study conducted by Krul-Poel and colleagues showed an adjusted significant difference between serum 25(OH)D and HOMA-IR (P<0.01). Li and colleagues also showed in their study on 25 women with PCOS and 27 controls an inverse correlation between 25(OH)D levels and BMI (P=0.033) and FAI (P=0.025) and a positive significant correlation with SHBG (P=0.038). However, the study by Elkholy and colleagues in Egypt in Aian Shams Maternity hospital conducted on 40 women with PCOS and 40 controls had shown no significant correlation between 25(OH) vitamin D and BMI (r=0.038, P=0.815) [16,17,31].

Thomson and colleagues in a review article stated that vitamin D levels have been negatively associated with IR (fasting insulin and HOMA-IR). However, this association disappeared as BMI was controlled. A study by Hahn and colleagues found that lower levels of 25OHD were associated with IR and obesity. One study showed that women with PCOS

with severe VDD were more insulin resistant, independently of BMI and WHR. Other observational studies have found relationships between markers of hyperandrogenism and vitamin D status. Women with hirsute have been shown to have lower 25OHD levels compared with BMI-matched control women (17 vs 29 ng/ml, respectively), and women with hirsute with PCOS have lower 25OHD levels compared with women with PCOS without hirsutism (21.4 vs 26.8 ng/ml, respectively). In women with PCOS, 25OHD levels have been positively associated with SHBG and negatively associated with the degree of hirsutism, FAI, and total testosterone, but this was no longer significant after adjusting for BMI and WHR. A similar result was found in a study by Wehr and colleagues. However, one study showed that women with PCOS with severe VDD were more insulin resistant, independently of BMI and WHR, and another showed that 25OHD levels were negatively correlated with BMI and HOMA-IR. Wehr and colleagues also using a multivariate regression analysis found that 25OHD levels were a significant and independent predictor for HOMA-IR along with BMI [29,31,33–35].We concluded that VDD is very common in women with PCOS and is associated with metabolic derangement, including IR, cardiovascular risk factors, as well as ovulatory dysfunction, infertility, and hirsutism.

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Conflicts of interest
There are no conflicts of interest.

References

1 Rusińska A, Płudowski P, Walczak M, Borszewska-Kornacka MK, Bossowski A, Chlebna-Sokół D, *et al.* Vitamin D Supplementation Guidelines for General Population and Groups at Risk of Vitamin D Deficiency in Poland-Recommendations of the Polish Society of Pediatric Endocrinology and Diabetes and the Expert Panel With Participation of National Specialist Consultants and Representatives of Scientific Societies-2018 Update. Front Endocrinol (Lausanne) 2018; 9:1–21.

2 Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266–281.

3 Verstuyf A, Geert C, Bouillon R, Mathieu C. Vitamin D: a pleiotropic hormone. Kidney Int 2010; 78:140–145.

4 Teede H, Misso ML, Costello MF, Dokras A, Laven J, Moran L, *et al.* Executive Summary. International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018; 6–16.

5 Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, *et al.* Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab 2006; 91:4237–4245.

6 Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod 2016; 31:2841–2855.

7 Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med 2010; 8:41.

8 Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005; 90:1929–1935.

9 Dokras A, Saini S, Gibson-Helm M, Schulkin J, Cooney L, Teede H. Gaps in knowledge among physicians regarding diagnostic criteria and management of polycystic ovary syndrome. Fertil Steril 2017; 107:1380–1386.

10 Gibson-Helm M, Teede H, Dunaif A, Dokras A. Delayed diagnosis and a lack of information associated with dissatisfaction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2017; 102:604–612.

11 Teede H, Misso ML, Costello MF, Dokras A, Laven J, Moran L, *et al.* Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod 2018; 33:1602–1618.

12 Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol 2013; 6:1–13.

13 Amowitz LL, Sobel BE. Cardiovascular consequences of polycystic ovary syndrome. Endocrinol Metab Clin North Am 1999; 28:439–458.

14 Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 2004; 89:2745–2749.

15 Ketel IJ, Serne EH, Ijzerman RG, Korsen TJM, Twisk JW, Hompes PGA, *et al.* Insulin-induced capillary recruitment is impaired in both lean and obese women with PCOS. Hum Reprod 2011; 26:3130–3137.

16 Elkholy SSH, Mostafa RA, Riad AA, AbouZaghla HM. Assessment of vitamin D levels in women with polycystic ovarian syndrome. Egypt J Hosp Med 2018; 70:594–600.

17 Krul-Poel YHM, Koenders PP, Steegers-Theunissen RP, Ten Boekel E, Wee MMT, Louwers Y, *et al.*.Vitamin D and metabolic disturbances in polycystic ovary syndrome (PCOS): A cross-sectional study. PLoS ONE 2018; 13:e0204748.

18 Krul-Poel YH, Snackey C, Louwers Y, Lips P, Lambalk CB, Laven JS, Simsek S. The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review. Eur J Endocrinol 2013; 169:853–865.

19 Varanasil LC, Subasinghe A, Jayasinghe YL, Callegaril ET, Garland SM, Gorelik A, *et al.* Polycystic ovarian syndrome: prevalence and impact on the wellbeing of Australian women aged 16–29 years. Aust N Z J Obstet Gynaecol 2018; 58:222–233.

20 Zawadzki J, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Given JR, Haseltine FP, Merriam GR, editors. Polycystic Ovary Syndrome. Boston, MA: Blackwell Scientific Publications; 1992. 377–384

21 Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81:19–25.

22 Tsilchorozidou T, Overton C, Conway GS. The pathophysiology of polycystic ovary syndrome. Clin Endocrinol (Oxf) 2004; 60:1–17.

23 Teede H, Misso ML, Costello MF, Dokras A, Laven J, Moran L, *et al.* Screening, diagnostic assessment, risk assessment and life-stage. International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018; 1:35–57.

24 Moini A, Shirzad N, Ahmadzadeh M, Hosseini R, Hosseini L, Sadatmahalleh SJ. Comparison of 25-hydroxyvitamin D and calcium levels between polycystic ovarian syndrome and normal women. Int J Fertil Steril 2015; 9:1–8.

25 Lakshman LR, Pillai BP, Lakshman R, Kumar H, Sudha S, Jayakumar RV. Comparison of vitamin D levels in obese and non-obese patients with polycystic ovarian syndrome in a South Indian population. Int J Reprod Contracept Obstet Gynecol 2013; 2:336–343.

26 Mazloomi S, Sharifi F, Hajihosseini R, Kalantari S, Mazloomzadeh S. Association between hypoadiponectinemia and low serum concentrations of calcium and vitamin D in women with polycystic ovary syndrome. ISRN Endocrinol 2012; 2012:949427.

27 Savastano S, Valentino R, Di Somma C, Orio F, Pivonello C, Passaretti F, *et al.* Serum 25-hydroxyvitamin D levels, phosphoprotein enriched in diabetes gene product (PED/PEA-15) and leptin-to-adiponectin ratio in women with PCOS. Nutr Metab (Lond) 2011; 8:84.

28 Hassan NE, El Orabi HA, Eid YM, Mohammed NR. Effect of 25-hydroxyvitamin D on metabolic parameters and insulin resistance in patients with polycystic ovarian syndrome. Mid East Fertil Soc J 2012; 17:176–180.

29 Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. Clin Endocrinol (Oxf) 2012; 77:343–350.

30 Wehr E, Trummer O, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. Eur J Endocrinol 2011; 164:741–749.

31 Li HW, Brereton RE, Anderson RA, Wallace AM, HO CK. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. Metabolism 2011; 60:1475–1481.

32 Kim JJ, Choi YM, Chae SJ, Hwang KR, Yoon SH, Kim MJ, *et al.* Vitamin D deficiency in women with polycystic ovary syndrome. Clin Exp Reprod Med 2014; 41:80–85.

33 Hahn S, Haselhorst U, Tan S, Quadbeck B, Schmidt M, Roesler S, *et al.* Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes 2006; 114:577–583.

34 Yildizhan R, Kurdoglu M, Adali E, Kolusari A, Yildizhan B, Sahin HG, *et al.* Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. Arch Gynecol Obstet 2009; 280:559–563.

35 Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber TR, *et al.* Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. Eur J Endocrinol 2009; 161:575–582.