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Supplementation of Vitamin D improves Insulin Resistance and Oxidative Stress in PCOS women. A Comprehensive Review

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Abstract

The aim of the present review was to evaluate the effect of vitamin D (VD) supplementation (alone or co-supplementation) on insulin resistance and oxidative stress in VD deficient polycystic ovary syndrome (PCOS) subjects. Using PubMed, Google Scholar and Web of Science, we searched for publications in the last 10 years regarding the role of VD in PCOS students. It was found that the prevalence of VD deficiency was higher in PCOS subjects in comparison to control. We compared the effects of supplementation of VD in PCOS patients on insulin resistance (IR) value and oxidative stress markers (MDA). It was found that VD as a co-supplement could significantly decrease fasting glucose concentrations and the HOMA-IR value. Further VD supplementation in patients with PCOS resulted in a significant improvement in high sensitivity C-reactive protein (hs-CRP), total antioxidant capacity (TAC), and malondialdehyde (MDA).

Key words: Vitamin D, PCOS, Insulin Resistance, Oxidative stress, C-reactive protein

Introduction

Polycystic ovary condition (PCOS) is the most common endocrine disorder in women of reproductive age, with a predominance of 6–10% in general population.[1] It is the most frequently recognized reason for anovulatory infertility and has been reported to be related to insulin resistance (IR), hyperinsulinemia and dyslipidaemia which are all risk factors for the metabolic disorder, type 2 diabetes mellitus, and cardiovascular disease.[2-4] Polycystic ovaries are characterized by the presence of at least eight sub capsular follicular cyst ≤10 mm and increased ovarian stoma seen under ultrasound. [5]

Vitamin D, a steroid hormone, is involved in calcium metabolism and bone structure and has a potential role in the prevention of many ailments including cancers, autoimmune disorders, hypertension, diabetes, and obesity. An inadequacy of vitamin D causes poor bone mineralization as well as has been involved in various persistent infections including diabetes, coronary heart disease, poor immunity, different malignant conditions, multiple sclerosis, rheumatoid arthritis, and hypertension. [6] Vitamin D exerts a significant role in the health and fertility for women and plays a physiologic role in reproduction including ovarian follicular development and luteinization through changing anti-mullerian hormone (AMH) signaling, follicle-stimulating hormone (FSH) sensitivity and progesterone production in human granulosa cells. In spite of its importance, research shows that 67-85% of women with PCOS are suffering from vitamin D deficiency.[7]

Vitamin D synthesis and metabolism

Vitamin D exists in two forms, vitamin D2 and vitamin D3. Vitamin D2 (ergocalciferol) originates from ergosterol of yeast, while vitamin D3 (cholecalciferol) is produced by the skin epidermal cells from 7-dehydrocholesterol in the presence of bright sunlight (UV-B). Then it undergoes hydroxylation in the liver by vitamin D-25- hydroxylase (25-OHase) to form 25-hydroxyvitamin D [25(OH)D], the circulating form of vitamin D. The 25(OH)D is the most consistent index of human vitamin D status. After being secreted into the blood, 25(OH)D circulates into the kidney and is converted into 1,25-dihydroxy-vitamin-D [1,25(OH)2D3], which is the most active form. The hydroxylation in the kidney could be terminated by 25(OH)D-24- hydroxylase (or CYP24A1), which is responsible for the degradation of 1,25(OH)2D3. [8]

Circulating 1,25(OH)2D3 exerts its biological function by binding to a nuclear receptor (vitamin D receptor, VDR) and forming a heterodimer with retinoid X receptor (RXR). This dimerization resulted in the regulation of gene transcription through VD responsive elements (VDRE) in the promoter regions of several VD target genes involved in bone and mineral metabolism, oxidative damage, chronic diseases and inflammation. [9]

Vitamin D and PCOS

Many recent reports indicate vitamin D deficiency is associated with reproductive complications and metabolic disturbances in PCOS patients.[9] The connection between VD levels and various PCOS symptoms, including insulin resistance (IR), anovulation and hirsutism has been reported in a few studies.[10] VD level was negatively associated with serum androgen level and VD treatment could reduce serum androgen and anti-Müllerian Hormone (AMH) levels. [11] Supplementation with VD improves endometrial thickness, which resulted in improvement of menstrual cycle and folliculogenesis in PCOS patients. [12]. It has been proposed that VD inadequacy and insufficient dietary calcium might be accountable for the irregularities associated with occurrence of PCOS. VD insufficiency build parathyroid hormone (PTH) production which is linked to PCOS, anovulatory cycle and increased testosterone. [13-15] The pathogenesis of PCOS has been connected with the VDR polymorphisms on luteinizing hormone (LH) and sex hormones binding globulin (SHBG) levels, testosterone levels, insulin resistance and serum insulin levels.[16] VD directs estrogen biosynthesis and aromatase gene articulation by balancing extracellular calcium homoeostasis. [17,13] In human ovarian tissue, estrogen and progesterone production is enhanced whereas testosterone production is reduced by VD which might be due to boosting of aromatase circulation. Therefore, VD inadequacy may give rise to PCOS symptoms. VD also plays a significant role in conceptive capacity because of the fact that VDRs present in the ovary, endometrium and placenta. [18,11] Further, VD insufficiency is related with calcium deregulation, which takes part in the advancement of follicular arrest in females with PCOS and end up with menstrual and fertility dysfunction. [19]

Several observational studies focused on the association between low vitamin D concentrations and PCOS. Many reports indicated a higher prevalence of VD inadequacy in PCOS women. [20-24] (Table 1). Higher prevalence of VD deficiency was found in PCOS women than control. [20-24] Kruel-poel et al found an association of VD deficiency with unfavourable lipid profile and higher IR status in PCOS subjects. [20] Similarly, Gokosmanoglu et al reported an inadequate VD was associated with higher androgen levels in VD deficient PCOS women. [23].

PCOS and IR

It is estimated that between 50 - 90% of women with PCOS exhibit insulin resistance.[25] Hyperinsulinemia, which develops as a compensatory response to insulin resistance, interacts synergistically with LH to activate *CYP17* (encoding P450c17α, a key enzyme in ovarian androgen biosynthesis) which in turn enhances the generation and release of androgens.[26] Within the ovary, insulin also promotes arrest of pre-antral follicle development.[27] In PCOS the PI3-kinase pathway is dysfunctional, resulting in various cellular responses to insulin and subsequent augmentation of steroidogenesis, manifested as metabolic dysfunction, hyperandrogenaemia and reproductive dysfunction. [28]

Role of vitamin D deficiency in insulin resistance

Vitamin D deficiency has been proposed to play an important role in the development of insulin resistance and the pathogenesis of type 2 DM by affecting insulin sensitivity or/and *β*-cell function. [29-30] Vitamin D directly effects insulin action by stimulating the expression of insulin receptors and thus enhancing insulin responsiveness for glucose transport, [31] or indirectly by regulating extracellular calcium influx through cell membranes [32] Moreover, VD deficiency may increase insulin resistance in an indirect way through the compensatory increase in PTH levels which has been reported to decrease insulin sensitivity by hampering insulin synthesis and secretion from β-cells. [28].

VD and IR and PCOS

Several reports indicate VD concentrations are negatively correlated with fasting and stimulated glucose, homeostatic model assessment–insulin resistance (HOMA-IR). Previous observational studies have reported that supplementation of VD have improved IR in PCOS subjects. [35-42] **(Table 2)** Significant improvement in IR indicated by HOMA-IR [39, 41], Matsuda ISI [40], QUICKI [37], improved fasting plasma glucsose [39,42] serum fasting insulin [37] were observed in the vitamin deficient PCOS subjects after vitamin D supplementation. Most of the interventions were 8 weeks [38-41], 12 weeks [35,37] and 24 weeks [42]. Most of the interventions were on weekly basis [35,35,37,39] and some were given daily [40,41].

Oxidative stress (OS) and PCOS

Oxidative stress (OS) results due to imbalance caused by increased generation of free radical above the physiological range, and their reduced clearance by the antioxidant mechanisms of cells [43]. Free radicals are active derivatives of either the oxygen molecule such as reactive oxygen species (ROS: hydroperoxyl, superoxide, hydrogen peroxide, and hydroxyl radicals or reactive nitrogen species (RNS). [43] The endogenous source of ROS includes leakage of activated oxygen during oxidative phosphorylation, from the detoxification reactions involving the liver cytochrome P-450 enzyme system, peroxisomal oxidases, NAD(P)H oxidases or XO. [44] Exogenous sources includes exposure to environmental pollutants, smoke; consumption of alcohol; exposure to ionizing radiation; microbial infections, etc. [45] Most biologic cells have an intrinsic defence mechanism involving various enzymes such as superoxide dismutase (SOD), catalase (CAT), and non-enzymatic antioxidants includes Glutathione (GSH) and vitamins such as E, C, and A. the ROS can directly oxidize proteins, lipids, and nucleic acids and produce toxic by-products leading to tissue dysfunction [46] or can indirectly damage cells by activating a variety of stress-sensitive intracellular signalling pathways such as Nf-*κ*b (nuclear factor kappa b), p38 MAPK (p38 mitogen-activated protein kinases), JNK/SAPK (stress-activated protein kinase/c-Jun NH(2)-terminal kinase), PKC (protein kinase C), AGE/RAGE (advanced glycation end product/receptor for AGE) [47].

Higher OS level is related with obesity, insulin resistance, hyperandrogenaemia, and inflammation.[48] Oxidative stress disturbs the pancreatic beta-cell function via several molecular mechanisms. Beta cells have a low capacity of the antioxidant defence system, hence, oxidative stress in beta cells is more prevalent. OS can also induce apoptotic processes in the pancreatic cells leading to death and loss of beta cells [49]. An overload of free radicals not only induce apoptotic processes in the pancreatic cells leading to death and loss of beta cells but also disturbs the beta-cell neogenesis [50]. Oxidative stress also impairs insulin signalling by inducing IRS-1 and IRS-2 serine phosphorylation, which in turn results in a disturbed IST [51]. OS induces the inflammatory responses involved in insulin resistance [52]

A lot of studies have reported that oxidative stress circulating markers like MDA, SOD, GPx etc. are significantly increased in patients with PCOS compared with the normal and are considered as a potential cause of PCOS pathogenesis. [53] OS and IR goes hand in hand. On one hand OS induces the inflammatory responses involved in insulin resistance. And on the other hand IR energizes OS since hyperglycaemia and higher levels of free unsaturated lipids leads to ROS creation [54].

Role of vitamin D in Oxidative Stress

Vitamin D is a powerful anti-oxidant that improves mitochondrial functions and prevents oxidative stress related protein oxidation, lipid peroxidation, and DNA damage.[55] Normal VD status downregulates many of the intracellular oxidative stress-related activities. However, suboptimal concentrations of serum 25(OH)D fail to control oxidative stress conditions, augment intracellular oxidative damage and the rate of apoptosis. VD modulates Nrf2 which is a key player in protecting cells against oxidative stress [56]. Calcitriol upregulates the expression of certain antioxidants and anti-inflammatory cytokines and thereby controls ROS levels. VD can also inhibit NF-κB, thus, reducing the production of free radicals and pro-inflammatory cytokines.[57-58] VD supplementation attenuates the deposition of advanced glycation end products (AGEs) and increases serum sRAGE in hemodialysis patients.This suggests that vit D3 treatment could play an anti-inflammatory role by altering the AGE-RAGE system. [59]. Further VD decreases C-Reactive proteins (CRPs) which are an indication of infection [60].

Compared with non-PCOS patients, the level of OS in PCOS patients is elevated, and the proportion of oxidants is higher than that of antioxidants. Malondialdehyde (MDA) is a product of ROS-induced lipid peroxidation, hence, can be used to detect OS. During OS, the antioxidant enzymes (SOD, CAT, GPx) in the body are continuously consumed, leading to a decrease in total antioxidant capacity (TAC) levels. Thus, MDA and TAC levels reflect the production of ROS.

In this present comprehensive review, it was found that VD supplementation improved GSH, MDA levels, TAC, in PCOS subjects [61-67]. **(Table 3)** Various studies have reported decrease in serum hs-CRP levels after vitamin D supplementation in patients with PCOS. [61, 63,66, 67]. Moreover, VD co-supplemented with calcium [61]. Evening primerose oil [65], magnesium, zinc and calcium [66], potassium and calcium [62] appeared to be effective in PCOS patients. Most of the studies had treatment period of 12 weeks [61-63] followed by 8 weeks in some [64-67].

Conclusion

The current review demonstrates that vitamin D inadequacy or insufficiency is prevalent in PCOS subjects and supplementation of VD in these PCOS patients resulted in an improvement

in the levels of oxidative stress parameters like MDA and CRP and improved TAC and insulin resistance as seen in improved HOMA-B.

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Table1. Prevalence of VD deficiency in PCOS subjects

Author	Study group	Outcome
Krul-Poel (2018) [20]	639	A compromised vitamin D status β = 0.78; 95% CI: 0.72–0.84, p < 0.01) was found in PCOS women Serum 25(OH)D was significantly lower in PCOS women compared to fertile controls (mean 25(OH)D of 49.0 nmol/l *versus* 64.5 nmol/l).
Davis et al (2019) [21]	137	Vitamin D deficiency occurred more frequently in PCOS cases (47.6%; P value=0.06)
Wang eta al (2020) [22]	169	prevalence rates of 25(OH)D deficiency and insufficiency were significantly higher in women with PCOS than in controls (54.4% vs. 37.7%, *P* < 0.01; 34.9% vs. 23.7%, *P* < 0.05).
Gokosmanoglu (2018) [23]	231	low 25(OH)D3 levels are associated with high androgen levels testosterone (r =−0.374) and DHEAS levels (r=−0.418); (all; p< 0.05) in women with PCOS.
Lone et al (2020) [24]	235	PCOS was independently associated with vitamin D deficiency Mean serum 25(OH)D concentration was lower in cases vs controls (17.4 vs 21.7 ng/ml, respectively; P < 0.001).

Table2. Supplementation of VD improves IR in PCOS subjects

Author	Dosage of VD	Intervention duration	Study group	Outcome of VD supplementation
Nazia Raja-khan(2014) [35]	12,000 IU/weekly	12 weeks	22	decreased 2-hour insulin and glucose and a protective effect on blood pressure. No effect on IR
Maktabi (2017) [36]	50,000 IU/weekly	12 weeks	70	decreased (FPG) (−3.1±7.3 vs. +0.5±6.3 mg/dl, p=0.02), insulin (−1.4±3.6 vs. +2.6±7.0 µIU/ml, p=0.004), HOMA-IR (−0.3±0.8 vs. +0.6±1.6, p=0.003), HOMA-B (−4.9±13.4 vs. +9.9±26.9, p=0.005), and increased QUICKI (+0.01±0.01 vs. −0.02±0.05, p=0.007)
Gupta et al (2017) [37]	60,000 IU/weekly	12 weeks	25	QUICKI (0.37 ± 0.04–0.394 ± 0.009, p = 0.001) (IR) (2.38 ± 4.88–1.00 ± 0.58, p = 0.003), serum fasting insulin (10.34 ± 20.00–5.00 ± 3.25, p = 0.021),
Abootorabi (2018) [39]	50,000 IU/weekly	8 weeks	36	improved the FPG (7.67 ± 7.66 versus 1.71 ± 7.50 mg/dL, p = .001), HOMA-B (129.76 ± 121.02 versus 48.32 ± 128.35, p = .014),
Karadag et al (2018) [40]	50,000 IU/weekly 1500 IU/daily	8 weeks	54	increased insulin sensitivity Matsuda ISI (r = 0.307; P < 0.01 in vitamin-D-deficient PCOS women
Karamali et al., 2018 [41]	200 IU/daily	8 weeks	55	decreases in serum insulin concentrations (−1.9±3.5 vs. +1.8±6.6 µIU/mL, P=0.01), HOMA-IR (−0.4±0.7 vs. +0.4±1.4, P=0.01), HOMA-B (−7.9±14.7 vs. +7.0±30.3, P=0.02) and a significant increase in QISCI (+0.01±0.01 vs. −0.008±0.03, P=0.01).
Trummer (2019) [42]	20,000 IU/weekly	24 weeks	123	Reduced plasma glucose during OGTT (mean treatment effect − 10.2 mg/dL; 95% CI − 20.2 to − 0.3; p = 0.045).

Table3. Supplementation of VD improves OS in PCOS subjects

Author	Only VD or with Co-Supplementation	Study subject	Intervention period	Dosage of VD	Outcome of VD supplementation
Foroozanfard et al (2015) [61]	VD + Ca (compared with calcium alone, vitamin D alone and placebo groups)	104	8 weeks	50, 000 IU/ weekly	Improved CRP (−948·3 *vs* 802·3, −383·8 &618·2 ng/ml, respectively, *P* = 0·04),MDA(−0·6 *vs* −0·5, −0·1 and 0·6 µmol/l, respectively, *P* = 0·009), TAC (35·2 *vs* 21·1, 22·5 and −153·8 mmol/l, respectively, *P* = 0·006) ,GSH(216·0 *vs* 3·9, −47·5 and −160·8 µmol/l, respectively, *P* = 0·001)
RAzavi at al (2016) [62]	Vit D +k+ calcium (compared with Placebo)	60	8 weeks	200 IU /daily	PlasmaTAC(+ 75.7±126.1 vs.−80.4±242.8 mmol/l, p=0.005) , and MDA (+ 0.03±0.6 vs.+1.4±2.4 µmol/l, p=0.005) levels.
Jamilian, 2017 [38]	VD (compared with Placebo)	60	12 weeks	1000 IU /daily 4000 IU /daily	hs-CRP (−0.7 ± 1.4 vs. −0.5 ± 0.9 and +0.5 ± 2.4 mg/L, respectively, *p* = 0.01), (TAC) (+130 ± 144 vs. +33 ± 126 and −36 ± 104 mmol/L, respectively, *p* < 0.001
Jamilian 2018 [63]	VD + omega -3 fatty acids Compared with placebo	60	12 weeks	50,000 IU/ 2 weekly	plasma TAC (+ 114.6 ± 122.2 vs. - 2.4 ± 168.2 mmol/L, *P* = 0.003), hs-CRP (−1.2 ± 1.9 vs. + 0.1 ± 0.7 mg/L, *P* = 0.001) and plasma malondialdehyde (MDA) concentrations (−0.4 ± 0.4 vs. + 0.2 ± 0.6 µmol/L, *P* < 0.001)
Nasri (2018) [65]	VD + EPO (compared with placebo)	60	12 weeks	(1,000 IU/day	Improved GSH (+62.7 ± 58.0 vs. −0.7 ± 122.7 µmol/L, *p* = 0.01),, and MDA (−0.4 ± 0.4 vs. +0.5 ± 1.8 µmol/L, *p* = 0.008) levels.
Maktabi et al (2018) [66]	VD + Zn+ Ca+Mg	70	12 weeks	400 Iu	CRP (−0.7 ± 0.8 vs. +0.2 ± 1.8 mg/L, *P* < 0.001), (−0.4 ± 0.3 vs. +0.2 ± 1.0 µmol/L, *P* = 0.01), TAC (+46.6 ± 66.5 vs. −7.7 ± 130.1 mmol/L, *P* = 0.04) hs-CRP,
Ostamohammadi et al (2019) [67]	VD+ probiotic compared with placebo	60	12 weeks	50,000 IU/ 2 weekly	CRP(β − 0.67 mg/L; 95% CI, − 0.97, − 0.38; *P* < 0.001) *(MDA)* levels (β − 0.25 µmol/L; 95% CI, − 0.40, − 0.10; *P* = 0.001), (TAC) (β 82.81 mmol/L; 95% CI, 42.86, 122.75; P < 0.001) and total glutathione (GSH) levels (β 40.42 µmol/L; 95% CI, 4.69, 76.19; *P* = 0.02),