RESEARCH ARTICLE

Vitamin D deficiency – A silent threat in expression of polycystic ovary syndrome: A case-control study

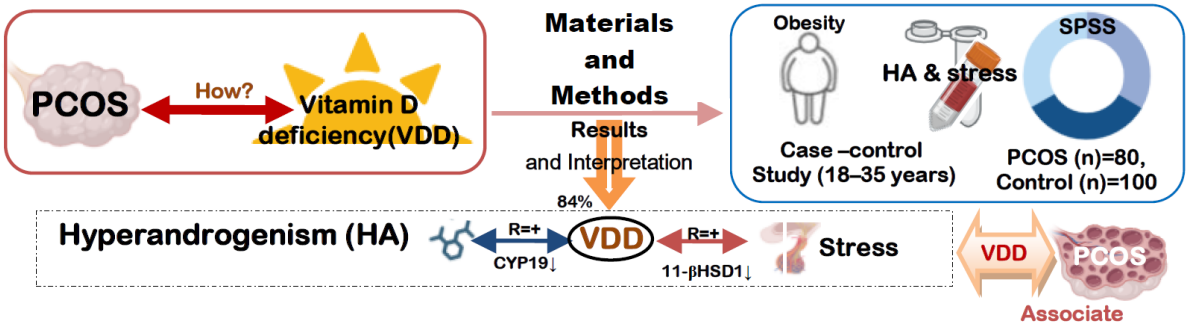
Sanchari Chakraborty[1,2], Tapan Kumar Naskar[3], Barnali Ray Basu[1*]

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a prevalent endocrinopathy in childbearing-aged women. Vitamin D deficiency (VDD) is now considered an alarming contributor to the pathophysiology of this syndrome. The levels of serum 25-hydroxy-vitamin D [25(OH)D] can potentially modulate features of PCOS, such as hyperandrogenism. *Materials and Methods*: The study was conducted with 80 patients with PCOS, and their gender, age (18–35 years), and ethnicity-matched 100 healthy controls, from July 2023 to March 2024, in and around Kolkata. With their informed consent, the anthropometric parameters were recorded for these volunteers. Free cortisol and sex hormone-binding-globulin (SHBG) were evaluated in the saliva of both groups of volunteers. Serum levels of vitamin D, estradiol (E2), LH, FSH, progesterone, and free testosterone were estimated. SPSS and Microsoft Office Excel were used for data analysis. *Results*: The prevalence of VDD and VD insufficiency (VDI) among the studied PCOS population was 84 and 11%, respectively. Vitamin D showed a significant inverse correlation with the LH/FSH ratio and free testosterone and an agonistic association with SHBG, estradiol, and progesterone. Additionally, it was found that VDD had a positive association with stress. The cortisol levels in the PCOS patients belonging to both the normal and overweight/obese categories were greater than those of BMI-matched control participants. *Conclusion*: VDD might be a silent risk factor in the penetrance of a multifaceted syndrome like PCOS.

Keywords: Polycystic ovary syndrome, vitamin D deficiency, hyperandrogenism, cortisol, stress.

Indian Journal of Physiology and Allied Sciences (2025); DOI: 10.55184/ijpas.v77i02.292 **ISSN:** 0367-8350 (Print)



Graphical Abstract

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifaceted and heterogeneous endocrine and metabolic disorder affecting adolescent girls and women of reproductive age. It manifests with a diversified range of symptoms, including irregular menstruation, hyperandrogenism (HA), polycystic ovarian morphology (PCOM), altered anti-Müllerian hormone (AMH) levels, insulin resistance (IR), obesity, and stress.[1-5] PCOS affects a significant portion of the woman population, with prevalence rates ranging from 28-75% in West Bengal, 4-20% in India, and 5-10% globally.[3-5] The disorder is characterized by multifactorial mechanisms and one of its hallmark features is the disruption of the hypothalamic-pituitary-ovarian (HPO) feedback loop, leading to increased gonadotropin-releasing hormone (GnRH) pulse frequency, hypersecretion of luteinizing hormone (LH), and an elevated LH-to-follicle-stimulating hormone (LH/FSH) ratio. This hormonal imbalance disrupts ovarian function, contributing to anovulatory infertility, follicular development issues, miscarriage, and increased risk of inflammation. Additionally, IR worsens the condition by reducing the synthesis of hepatic sex hormone-binding globulin (SHBG), which increases

[1]Clinicogenomics Lab, Sir Surendranath Banerjea Advanced Research Centre, Department of Physiology, Surendranath College, University of Calcutta, Kolkata, West Bengal-700009, India.

[2]Department of Physiology, Vidyasagar Metropolitan College, University of Calcutta, Kolkata, West Bengal, India.

[3]Department of Gynaecology and Obstetrics, Medical College, Kolkata, West Bengal-700073, India.

***Corresponding author:** Barnali Ray Basu, affiliation, Email: braybasu@gmail.com

How to cite this article: Chakraborty S, Naskar TK, Basu BR. Vitamin D deficiency – A silent threat in expression of polycystic ovary syndrome: A case-control study. *Indian J Physiol Allied Sci* 2025;77(2):14-20.

Conflict of interest: None

Submitted: 05/07/2024 **Accepted:** 19/01/2025 **Published:** 20/06/2025

free testosterone levels and perpetuates the hormonal imbalance.[3-8] Furthermore, PCOS affects the expression of proteins involved in DNA repair, mitochondrial metabolism, cell signaling, and apoptosis, impairing endometrial receptivity and fertility in both ovulatory and anovulatory women.[3-8] Stress is another critical factor influencing the development and severity of PCOS. Women with PCOS often experience stress due to anxiety, peer pressure, unhealthy dietary habits, and socioeconomic challenges.[3,8] Elevated stress levels affect the hypothalamic-pituitary-adrenal (HPA) axis, leading to cortisol dysregulation, which further indulges PCOS symptoms. Depending on the duration of stress, cortisol can either exacerbate or alleviate obesity and inflammation—two key features of PCOS.[1,5]

An emerging issue in PCOS management is the high prevalence of vitamin D deficiency (VDD), which affects between 67 and 85% of women with PCOS.[7] Vitamin D plays a crucial role in regulating reproductive health by influencing ovarian follicle development, steroidogenesis, and AMH levels. It also affects transforming growth factor-beta 1 (TGF-β1) activity and facilitates the mitigation of IR and dyslipidaemia. Adequate vitamin D levels improve ovarian reserve, follicle development, and androgen biosynthesis, reducing hyperandrogenism and improving overall ovarian function.[8-11] Vitamin D exerts its effects by binding to the vitamin D receptor (VDR) in reproductive tissues such as ovarian granulosa and theca cells, the uterine endometrium, and the HPO axis.[7,12,13] Additionally, vitamin D regulates the expression of cytochrome P450 enzymes (*CYP11A*, *CYP17*, and *CYP19*), which are involved in ovarian steroidogenesis.[14,15] The *AMH* gene contains a vitamin D response element (VDRE), highlighting the role of vitamin D in controlling AMH-associated follicular development.[8] Vitamin D also plays an important role in glucose metabolism in women with PCOS. By regulating glucose transporter type 4 (GLUT4) and calcium transport, vitamin D enhances insulin sensitivity, thereby helping to manage insulin resistance (IR).[8] Furthermore, it promotes lipolysis and regulates fat distribution, thereby modulating body mass index (BMI), a common indicator of obesity in PCOS.[2,16,17] Vitamin D also enhances progesterone activity through genomic and non-genomic pathways, affecting key components of the progesterone signaling cascade, including PGRMC1, PGRMC2, and nuclear receptors.[16] Moreover, vitamin D inhibits the transformation of bone marrow-derived mesenchymal stem cells (BM-MSCs) into adipocytes by regulating proteins in the Wnt signaling pathway, such as Dickkopf-1 (DKK1) and secreted frizzled-related protein 2 (SFRP2).[17] VDD also affects cortisol metabolism. By inhibiting the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), vitamin D helps lower cortisol levels, which may play a crucial role in managing the stress-related exacerbation of PCOS symptoms. Given the complex interplay between cortisol dysregulation, stress, and polycystic ovary syndrome (PCOS), vitamin D is a vital component in symptom management.[5] The global prevalence of VDD ranges from 20 to 60%, while in India, the rates are higher, between 34.2 to 98.8%, with 51 to 93% of women in West Bengal affected.[9,18,19] Modern lifestyle factors such as reduced sun exposure, sedentary habits, and poor diet contribute to VDD, further worsening PCOS symptoms like menstrual irregularities, ovarian dysfunction, and hyperandrogenism.[20,21] In India, despite the availability of sunlight, genetic factors such as variations in *VDR*, VD-binding protein (*VDBP*), and vitamin D metabolism enzymes (*CYP11A1*, *CYP2R1*, and *CYP24A1*) contribute to the high prevalence of VDD and its association with PCOS.[22-24] In West Bengal, a region known for its diverse ethnic and cultural background, obesity and PCOS-related complications are prevalent. According to the National Family Health Survey, 30% of women in Kolkata are classified as obese, and diabetes and cardiovascular diseases, both of which are linked to PCOS, are major health concerns in the region.[25] However, there is a lack of research on vitamin D levels and their association with PCOS severity among the ethnic population in West Bengal. Emerging studies suggest that VDD significantly influences PCOS manifestations by abnormally modulating ovarian steroidogenesis and menstrual rhythm (Figure 1). A previous study identified a complex relationship between VDD, IR, and altered AMH status in PCOS.[8] However, the precise mechanism of vitamin D's pleiotropic effects on PCOS remains unclear. This study aimed to investigate the impact of VDD on PCOS in the ethnic population of West Bengal to inform tailored interventions to reduce the penetrance and severity of the syndrome.

MATERIALS AND METHODS

Study Design and Sample Size

Patients visiting the outpatient Department (OPD) of Gynaecology and Obstetrics at the Medical College, Kolkata, West Bengal, and their attendants have been approached for enrollment in the study. Volunteers from patients diagnosed with PCOS (n = 80, age: 18 to 35 years) with complications in menstruation and/or fertility, their gender, and age-matched healthy control (n=100) were included in the current case-control observational study. The participants were given the personal and societal relevance of the study. The required written consents were obtained from the volunteers through

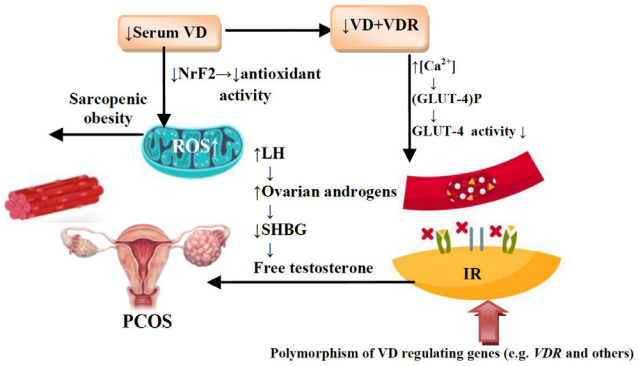


Figure 1: The keystone impact of VDD (vitamin D deficiency) on manifestations of PCOS (polycystic ovary syndrome)

predesigned and preapproved trilingual consent forms. Necessary ethical norms were observed and the study was approved by the Calcutta University Institutional Ethics Committee (CUIEC/03/40/2022-23), and Medical College and Hospital, Kolkata (MC/KOL/IEC/NON-SPON/1275/02/22). The collection of saliva samples and data was conducted in the outpatient department (OPD) of Gynaecology and Obstetrics at the hospital from July 2023 to March 2024. Midway through the study, a few respondents dropped out of the study (Figure 2). The EPITOOLS (Fremantle, Australia), an epidemiological calculator, has been used to calculate the sample size [n=180; inputs: expected proportion in controls: 0.05, odds ratio (OR): 4, confidence interval (CI): 0.95, and power: 0.8] in the present case-control observational study.[8,26]

Subject Selection

Case group

The Rotterdam criteria [European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM)], 2003, were used to confirm the PCOS diagnosis in volunteers participating in the study. Two of the three criteria were used for diagnosis of the syndrome: [a] an oligomenorrheic (≥35 days) or amenorrheic (>3 months) intermenstrual interval, [b] gynecological ultrasound of PCOM (number: >12, size: 2 to 9 mm, and ovarian volume: >10 cm^3), and [c] biochemical and/or clinical hyperandrogenism.[1,27,28] Women with intellectual and/or physical disabilities, pregnancy, and continued lactation were ruled out of the study. Virilizing tumors, prolactinoma, congenital adrenal hyperplasia (CAH), and Cushing syndrome simulating PCOS were also disregarded as potential etiologies.[1,27] Individuals with medical histories of oncology, endocrine medication, or contraceptives, present or in the past and implausible calorie intakes (≤500 or ≥4500 kcal/day) were also excluded.[5,29]

Control group

The healthy control participants had menstrual regularity, no evidence of biochemical and/or clinical manifestations of hyperandrogenism, no abnormally altered endocrine profile, no feature of PCOS, and neither a history of an unbalanced diet nor drug ingestion.[8,28-31]

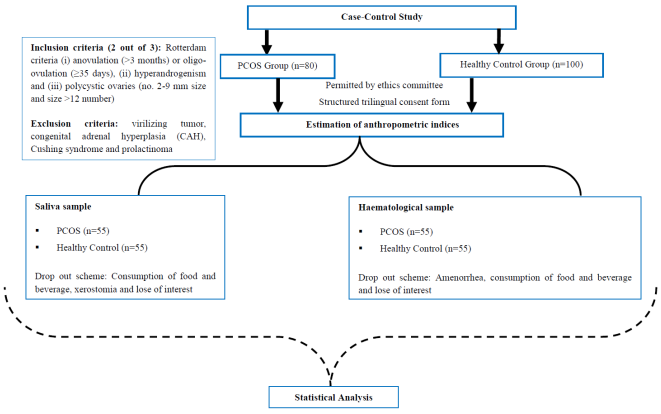


Figure 2: Flow chart for patient enrolment and dropout scheme

Evaluation of Anthropometric Indices

The height in centimeters of the barefoot participant was estimated by using a portable ultrasonic-based digital stadiometer (make: EasyCare EC1800, India). BMI (kg/m^2), body fat content (%), visceral fat, and skeletal muscle mass in whole-body (%) of the least-clad individuals were measured based on bioelectrical impedance analysis using a body composition monitor (model: OMRON-HBF-375, Karada Scan, Kyoto, Japan).[1]

Biochemical Assay

Collection of saliva

The participants were advised to rinse their mouths with water and abstain from food and beverages (except water) for one hour before collecting their saliva. After that, the individuals were instructed to sit comfortably in an upright position and tilt their heads forward to collect the whole saliva samples in the same time interval of day by the passive drool method in ice-chilled graduated cryovials (1.80 ml) through a saliva collection aid (Salimetrics, Item No. 5016.02, Carlsbad, United States). Subsequently, the saliva-filled cryovials were immediately transferred to a mini-cooler and then stored in a -20°C freezer until further assay.[1]

Enzyme-linked Immunosorbent Assay (ELISA) of Free Cortisol and Sex-Hormone-Binding-Globulin (SHBG)

The salivary active free cortisol was quantified by using a competitive ELISA kit (make: LDN; Germany, SA E-6000). The normal reference ranges (ng/ml) were in the morning 1.6 to 9.2. In the midday 0.9 to 6.9, and in the afternoon, 0.6 to 3.6, and the intra- and inter-assay CV% were 4.1 to 7.1 and 4.2 to 9.1, respectively. The salivary SHBG concentration was measured using a sandwich ELISA kit (make: MyBioSource, MBS2701743; San Diego, USA). The detection range is 31.2-2000 pg/ml, and the intra- and inter-assay CV% were <10 and <12, respectively. First, the saliva samples were centrifuged for 20 minutes at 4°C at 1000×g, and the supernatants were used in the subsequent stages of the assay. The salivary concentrations of cortisol and SHBG were evaluated spectrophotometrically by absorbance at the 450nm wavelength in a microplate reader [make: Bio-rad, Model No. iMark (Microplate) reader, SL No. 10095, California, United States].

Estimation of haematological parameters

The blood samples of the PCOS patients were collected by vein puncture after a 12-hour fast in the morning of the early follicular phase (the second or third day of menstruation). Blood (serum) 25-hydroxy-vitamin D [25(OH)D] concentration [reference ranges (ng/ml): <20 (VDD), 20 to <30 [vitamin D insufficiency (VDI)] and ≥30 [vitamin D sufficiency (VDS)] of the participants was evaluated by the chemiluminescent microparticle immunoassay (CMIA) method and the Alinityi (Abbott) system [assay CV (intra=3.66–6.56% and inter=4.19–7.01%)]. The contents of estradiol (E2), LH, and

Table 1: Analysis of exposure of inadequate vitamin D levels (Exposed) variants [VDD <20 ng/mL, VDI 20 to <30 ng/mL] and vitamin D sufficiency (not Exposed) variants [VDS≥30 ng/mL] in case (n=55) and control (n=55) groups

Vitamin D variants	*Exposure status*	*Case (PCOS)*	*Control*
VDD/VDI <30 ng/mL	Exposed	52	44
VDS ≥30 ng/mL	Not exposed	3	11

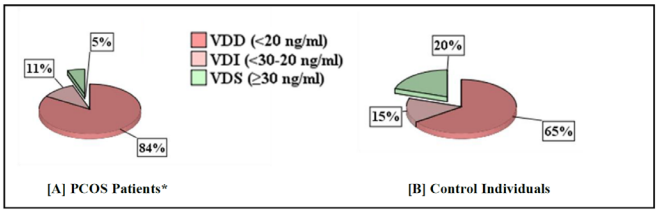


Figure 3: Prevalence of vitamin D deficiency (VDD) and insufficiency (VDI) in [A] PCOS patients (n = 55), and [B] control individuals (n = 55). VDS = vitamin D sufficiency, * indicates significantly ($p < 0.05$) lower vitamin D levels in PCOS patients relative to control individuals

FSH (method: electrochemiluminescence and system: cobas e 411); progesterone (P4, method: enzyme-linked fluorescent assay and system: MINIVIDAS), and free testosterone (method: ELISA) were measured from the drawn blood samples of the participants. The LH/FSH ratio was calculated by using the standard formula.[8]

Statistical Analysis

Statistical software for the social sciences (SPSS, IBM Corp., version 20, Chicago, Illinois, United States) was used to analyze the collected data. The asymmetric distribution pattern was determined by the Shapiro-Wilk (S-W for cortisol levels) and Kolmogorov-Smirnov (K-S for visceral fat, BMI, body fat content, and skeletal muscle-whole body) tests. Independent samples-t test and stacked-histogram (evaluation of different dispersion patterns of visceral fat, BMI, body fat content, and skeletal muscle-whole body in PCOS and control groups), pie chart (categorization of PCOS and control individuals determined by vitamin D levels), bivariate Pearson correlation [evaluation of the association between 25(OH)D with anthropometric parameters, and LH/FSH ratio, free testosterone, SHBG, E2, progesterone, and free cortisol] and population pyramid histogram (cortisol distribution based on BMI levels). Microsoft Office Excel (.xlsx; 2007, Redmond, Washington; United States) was used to evaluate exposure [OR (Odds Ratio) and 95% CI (Confidence Interval)] of vitamin D levels in the study population. The statistical significance was considered at the threshold of $p < 0.05$. The technical error was within the limit.[1]

RESULTS

Odds of exposure [inadequate vitamin D level (<30 ng/mL): VDD/VDI] were higher in PCOS (case), compared with those in the control group (OR = 4.33; 95% CI =1.14, 16.52) (Table 1). In the current study, it was found that PCOS individuals have

Table 2: Correlation between 25-OH vitamin D (ng/mL) and different obesity indices in case-control group

Obesity indices	*R scores*	
	PCOS Patients (n = 55)	*Healthy control (n = 55)*
Visceral fat	-0.023	-0.205
BMI (kg/m^2)	-0.013	-0.002
Body fat (%)	-0.063	-0.167
Skeletal muscle whole body (%)	0.059	0.216

a significantly ($p = 0.008$) lower level of vitamin D [VDD (11.67 ± 2.76), VDI (24.37 ± 2.75) and VDS (39.07 ± 10.94)] than the control individuals [VDD (12.2 ± 2.68), VDI (24.73 ± 2.43) and VDS (39.87 ± 3.38)] (Figure 3 A and B).

VDD represented a direct association with visceral fat (p = 0.870 and 0.134), BMI (p = 0.870 and 0.989), and body fat (p = 0.927 and 0.223) and an inverse relationship with skeletal muscle mass-whole body ($p = 0.669$ and 0.113) in both PCOS and control groups (Table 2).

In the PCOS patients, visceral fat was asymmetrically (K-S = 0.000) distributed, and BMI (K-S = 0.091), body fat content (K-S = 0.200), and skeletal muscle mass-whole body (K-S = 0.194) were normally distributed (Figure 4 I–IV). In the control group, visceral fat and skeletal muscle mass-whole body were asymmetrically (K-S = 0.000) distributed, and BMI (K-S = 0.200) and body fat content (K-S = 0.194) were normally distributed (Figure 4 I–IV). Interestingly, visceral fat (7.26 ± 5.02 and 4.99 ± 3.89, p = 0.001), BMI (25.92 ± 5.25 and 23.64 ± 4.79, p = 0.003) and body fat content (32.90 ± 5.37 and 30.61 ± 5.79, p = 0.007) were higher in the PCOS group than in the control subjects, whereas skeletal muscle mass-whole body (24.30 ± 2.05 and 25.47 ± 4.37, $p = 0.028$) showed an antagonistic relationship (Figure 4 I to IV).

The cortisol was asymmetrically distributed in both normal (BMI ≤ 25 kg/m^2, S-W = 0.011) and overweight/obese (>25 kg/m^2, S-W = 0.008) PCOS patients (Figure 5A. I and II). It was also found that PCOS patients (18.20 ± 4.39 and 18.75 ± 3.65) have a higher cortisol level than their BMI [normal (p = 0.026) and overweight/obese (p = 0.083)]-matched control individuals (15.70 ± 4.42 and 16.50 ± 4.12) (Figure 5A I and II). In the PCOS group, overweight/obese patients suffered from higher cortisol levels than the women with normal BMI (Figure 5A I and II). Furthermore, it was found that vitamin D has an inverse relationship with cortisol levels in both PCOS (p = 0.981) and control individuals ($p = 0.895$) (Figure 5B I and II). It was found that in the PCOS population, vitamin D was inversely correlated with the LH/FSH ratio ($p = 0.013$) and free testosterone (p = 0.000), and an affirmative association (p = 0.000) was seen with SHBG, E2, and progesterone (Figure 6. A to E).

DISCUSSION

7-dehydrocholesterol, a precursor to cholecalciferol, present in the skin, absorbs UVB radiation (290–315 nm) from

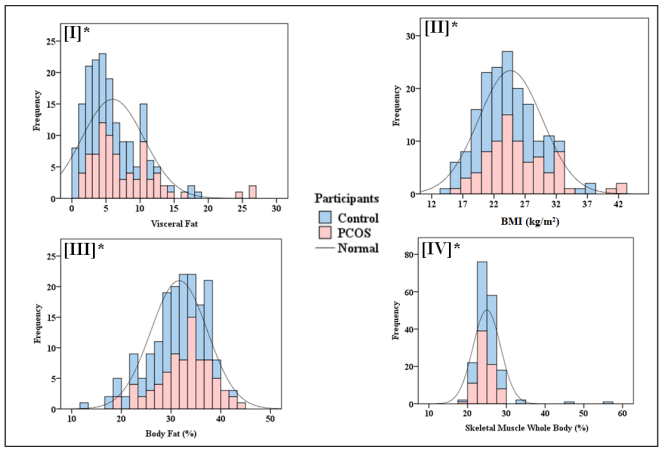


Figure 4: Comparison in [I] visceral fat and [II] BMI, [III] body fat content and [IV] skeletal muscle-whole body between the PCOS (n = 80) patients and control (n = 100) group. * Significantly (p <0.05) altered in PCOS patients relative to control individuals

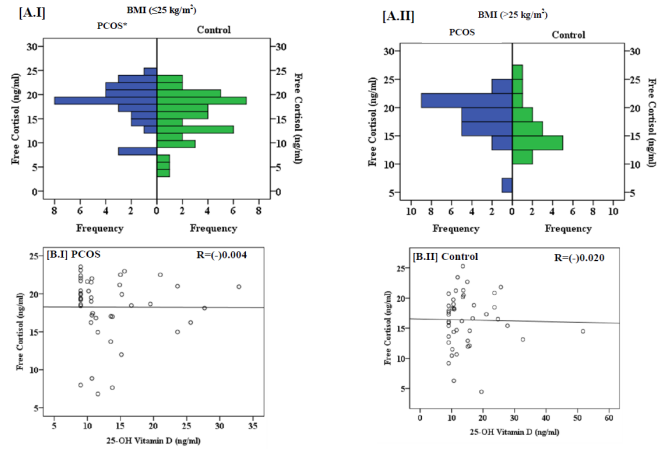


Figure 5: Comparison of free cortisol levels between PCOS and control participants in [A.I] BMI [≤25 kg/m^2, PCOS (n = 31) and control participants (n = 40)], and [A.II] BMI [>25 kg/m^2, PCOS (n = 24) and control participants (n = 15)], and correlation between vitamin D and free cortisol in [B.I] PCOS patients (n = 45), and [B.II] control individuals (n = 45). * Significant at p <0.05, VDS = vitamin D sufficiency

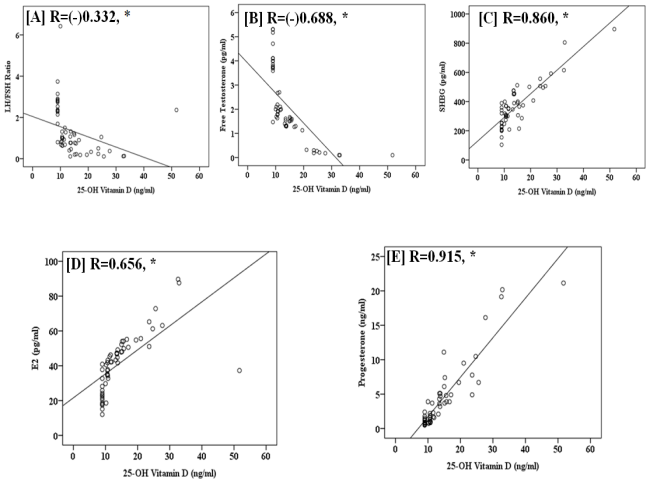


Figure 6: Correlation study between vitamin D and [A] LH/FSH (luteinizing hormone/follicle stimulating hormone) ratio [B] free testosterone, [C] SHBG (sex-hormone-binding globulin), [D] E2 (estradiol), and [E] progesterone in PCOS study population (n = 55). * Significant at p <0.05

sunlight, facilitating its conversion into provitamin D3, which subsequently undergoes isomerization to form vitamin D3, commonly referred to as the sunshine vitamin.[20,32,33] However, factors such as limited sun exposure, sedentary behavior, and inadequate dietary habits can contribute to VDD and obesity (Table 1).[20,32,33] Furthermore, experimental evidence from VDR$^{-/-}$ rodents suggests that VDD facilitates adipose tissue generation through adipogenesis from mesenchymal stem cells by suppressing Wnt/β-catenin signaling.[17] The VDD-associated distorted crosstalk can potentially alter the HPO axis-mediated ovarian steroidogenesis, which manifests as PCOS (Figure 3 and Table 2).[2,12,13,34] In our previous study, it was found that stress played a pivotal role in the manifestations of PCOS, where cortisol levels were higher in PCOS patients than those of their ethnicity-matched control individuals.[1] The present study (Figures 4 and 5) demonstrates that vitamin D exhibits antagonistic effects on cortisol, likely through its inhibitory action on the 11-βHSD1 enzyme, a key regulator of cortisol level.[5]

VDD is intricately interrelated with some alarming characteristics of PCOS, such as impairment of folliculogenesis-associated multifollicular ovaries and gonadal insufficiency-interlinked hyperandrogenism.[15,35] Kinuta et al. reported that a null mutant of *VDR* potentially reduces the expression of the aromatase gene (*CYP19*), which leads to suppression of E2 levels and elevated testosterone-associated hyperandrogenism.[35] Additionally, the low levels of vitamin D further decline the activity of GLUT4, which triggers a hyperinsulinemia-induced reduction in hepatic SHBG secretion.[36,37] In our previous observation, it was found that VDD indulges IR expression.[2] Vitamin D regulates the secretion and sensitivity of insulin in both non-genomic and genomic ways. It activates protein kinase-A (PKA), synthesis of phospholipase-C (PLC), and diacylglycerol (DAG), causing enhanced intracellular Ca^{2+}-induced insulin secretion. This upliftment of insulin genes via cAMP-responsive element binding protein (CREB) critically regulates the efficiency of insulin transcription, exocytosis, glucose sensing, and the survival of pancreatic β-cell.[8] This excess insulin triggers hyperandrogenism by stimulating LH-associated hypersecretion of androgens from the ovaries.[6] VDD can trigger hyperactivity of LH by abnormally modifying the mitochondrial and microsomal CYP family enzymes, which often manifest as an increased LH/FSH ratio (Figure 6. A to D).[15] The deficiency of vitamin D fastens its stimulatory effect on sections of ovarian cells and subsequent cell signaling activity of progesterone by altering the functionality of P4 membrane receptors, mPRα,β,γ and PGRMCs.[16,38] It was evident from the present study that vitamin D has an antagonistic effect on progesterone levels too (Figure 6.E).

This study highlights the potential role of VDD in contributing to hyperandrogenism, stress, and obesity in women with PCOS from West Bengal, India. Our previous findings identified vitamin D as a key regulator of AMH-mediated activities, further emphasizing its importance in PCOS pathophysiology. Given that vitamin D is a modifiable factor, treatment strategies with personalized vitamin D supplementation may offer a promising avenue for improving PCOS prognosis. However, to fully elucidate the mechanisms underlying the relationship between VDD and PCOS manifestations, further research is needed. Larger studies exploring the genetic polymorphisms of the vitamin D receptor (*VDR*), vitamin D-binding protein (*VDBP/GC*), and the *CYP* family genes involved in vitamin D metabolism, along with their impact on ovarian steroidogenesis, will be crucial in bridging the current knowledge gaps. These insights could ultimately contribute to more effective and targeted interventions for PCOS management.

ACKNOWLEDGMENTS

The authors are grateful to all women participants in this study. The authors are also thankful to Scientific Clinical Laboratory Pvt. Ltd. for providing technical support. The study is funded by the Department of Science & Technology and Biotechnology, Government of West Bengal (DSTBT-WB, Grant no. ST/P/S&T/9G-5/2018).

REFERENCES

1. Basu BR, Chowdhury O, Saha SK. Possible link between stress-related factors and altered body composition in women with polycystic ovarian syndrome. *J Hum Reprod Sci.* 2018;11(1):10-18. DOI: 10.4103/jhrs.JHRS_78_17.
2. Basu BR, Chakraborty S, Samaddar A, Pal R, Saha SK, Das N. Vitamin D and pathophysiology of polycystic ovary syndrome: a case–control study. *J Diabetol.* 2023;14(2):84-89. DOI: 10.4103/jod.jod_122_22.
3. Dhar S, Mondal KK, Bhattacharjee P. Influence of lifestyle factors with the outcome of menstrual disorders among adolescents and young women in West Bengal, India. *Sci Rep.* 2023;13(1):1-11. DOI: 10.1038/s41598-023-35858-2.
4. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. *Appl Clin Genet*. 2019;12:249-60. DOI: 10.2147/TACG.S200341.
5. Al-Dujaili EA, Munir N, Iniesta RR. Effect of vitamin D supplementation on cardiovascular disease risk factors and exercise performance in healthy participants: a randomized placebo-controlled preliminary study. *Ther Adv Endocrinol Metab.* 2016;7(4):153-65. DOI: 10.1177/2042018816653357.
6. Yau TT, Ng NY, Cheung LP, Ma RC. Polycystic ovary syndrome: a common reproductive syndrome with long-term metabolic consequences. *Hong Kong Med J.* 2017;23(6):622-34. DOI: 10.12809/hkmj176308.
7. He C, Lin Z, Robb SW, Ezeamama AE. Serum Vitamin D levels and polycystic ovary syndrome: a systematic review and meta-analysis. *Nutrients.* 2015;7(6):4555-77. DOI: 10.3390/nu7064555.
8. Chakraborty S, Naskar TK, Basu BR. Vitamin D deficiency, insulin resistance, and antimüllerian hormone level: a tale of trio in the expression of polycystic ovary syndrome. *F S Sci.* 2024;5(3):252-64. DOI: 10.1016/j.xfss.2024.06.002.
9. Ghosh J, Chaudhuri D, Saha I, Chaudhuri AN. Prevalence of metabolic syndrome, vitamin D level, and their association among elderly women in a rural community of West Bengal, India. *Medical J Dr. D.Y. Patil Vidyapeeth.* 2020;13(4):315-20. DOI: 10.4103/mjdrdypu.mjdrdypu_229_19.
10. Palomba S, Daolio J, Sala GBL. Oocyte competence in women with polycystic ovary syndrome. *Trends Endocrinol Metab*. 2017;28(3):186-98. DOI: 10.1016/j.tem.2016.11.008.
11. Varbiro S, Takacs I, Tuu L, Nas K, Sziva RE, Hetthéssy JR, Torok M. Effects of vitamin D on fertility, pregnancy and polycystic ovary syndrome-a review. *Nutrients.* 2022;14(8):1-17. DOI: 10.3390/nu14081649.
12. Keane KN, Cruzat VF, Calton EK, Hart PH, Soares MJ, Newsholme P, Yovich JL. Molecular actions of vitamin D in reproductive cell biology. *Reproduction.* 2017;153(1):R29-R42. DOI: 10.1530/REP-16-0386.
13. Liu Z. Association between 25-hydroxyvitamin D concentrations and pubertal timing: 6–14-year-old children and adolescents in the NHANES 2015–2016. *Front Endocrinol.* 2024;15(1394347):1-8. DOI: 10.3389/fendo.2024.1394347.
14. Ballesteros AIR, Meza MRM, Lamotte BV, Rojas IP, Mosso UDLC. Association of vitamin D metabolism gene polymorphisms with autoimmunity: evidence in population genetic studies. *Int J Mol Sci.* 2020;21(24):1-24. DOI: 10.3390/ijms21249626.
15. Chaudhary H, Patel J, Jain NK, Joshi R. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. *J Ovarian Res.* 2021;14(1):1-21. DOI: 10.1186/s13048-021-00879-w.
16. Rafiee M, Naseri M, Fakhrabadi MA, Motamedi N, Ghahiri A, Mehrabian F, Rezaei A. Vitamin D3 induces the expression of membrane progesterone receptors (mPRs) on naive CD^{4+} T lymphocyte cells in women of reproductive age. *Int Immunopharmacol*. 2019; 72:55-61. DOI: 10.1016/j.intimp.2019.03.053.
17. Pajor IS, Miazek K, Selmi A, Balcerczyk A, Sliwinska A. The action of vitamin D in adipose tissue: is there the link between vitamin d deficiency and adipose tissue-related metabolic disorders? *Int J Mol Sci.* 2022;23(2):1-35. DOI: 10.3390/ijms23020956.
18. G R, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. *Nutrients.* 2014;6(2):729-75. DOI: 10.3390/nu6020729.
19. Ismail NH, Mussa A, Khreisat MJA, *et al*. The global prevalence of vitamin D deficiency and insufficiency in patients with multiple myeloma: a systematic review and meta-analysis. *Nutrients.* 2023;15(14):1-21. DOI: 10.3390/nu15143227.
20. Patwardhan VG, Mughal ZM, Chiplonkar SA, *et al*. Duration of casual sunlight exposure necessary for adequate vitamin D status in Indian men. *Indian J Endocrinol Metab.* 2018;22(2):249-55. DOI: 10.4103/ijem.IJEM_473_17.
21. Morgante G, Darino I, Spano A, *et al*. PCOS physiopathology and vitamin D deficiency: biological insights and perspectives for treatment. *J Clin Med.* 2022;11(15):1-14. DOI: 10.3390/jcm11154509.
22. Anjana RM, Pradeepa R, Das AK, *et al*. Physical activity and inactivity patterns in India - results from the ICMR-INDIAB study (Phase-1) [ICMR-INDIAB-5]. *Int J Behav Nutr Phys Act.* 2014;11(1):1-11. DOI: 10.1186/1479-5868-11-26.
23. Dasgupta S, Dutta J, Annamaneni S, Kudugunti N, Battini MR. Association of vitamin D receptor gene polymorphisms with polycystic ovary syndrome among Indian women. *Indian J Med*

Res. 2015;142(3):276-85. DOI: 10.4103/0971-5916.166587.
24. Pal R, Choudhury S, Kumar H, Dey S, Das N, Basu BR. Vitamin D deficiency and genetic polymorphisms of vitamin D-associated genes in Parkinson's disease. *Eur J Neurosci*. 2023;58(5):3362-77. DOI: 10.1111/ejn.16098.
25. Indus Health Plus Doctors Team, West Bengal Health Statistics, https://www.indushealthplus.com/west-bengal-health statistics.html#: ~:text=About%2030%25%20of%20the%20 women, between%203.5%25%20%2D%205.7%25.
26. Villarta RL, Asaad AS. Sample size determination in an epidemiologic study using the EPI tools web-based calculator. *Acta Med*. 2014;48(1):42-6. DOI: 10.47895/amp. v48i1.1192.
27. Mallick P, Chakraborty S, Pal R, Samaddar A, Saha SK, Basu BR. Readdressing pathophysiology of polycystic ovary syndrome in post-COVID new normal era: a pilot study. *BOHR Intl J Gynecol*.2023;2(1):1-9. DOI: 10.54646/bijg.2023.01.
28. Basu BR, Chakraborty S, Saha SK, Das N. Evaluation of association of anthropometric indices with stress response in PCOS population. *Int J Sci Res*. 2020;9(10):79-83. DOI: 10.36106/ijsr/1812031.
29. Lafuente FN, Gonzalo JJA, Ferrer MLS, *et al*. Fat intake pattern in women with polycystic ovary syndrome. *Reprod Biomed Online*.2022;44(1):93-103. DOI: 10.1016/j.rbmo.2021.08.015.
30. Chakraborty I, Chattopadhyay R, Haldar R, Chakravarty B, Chakraborty P. Hyperhomocysteinemia, the player behind the curtain: a new insight into polycystic ovary syndrome. *Indian J Physiol Allied Sci*. 2022;74(3):12-7. DOI: 10.55184/ijpas. v74i3.43.
31. Jamil AS, Alalaf SK, Tawil NGA, Shawaf TA. A case-control observational study of insulin resistance and metabolic syndrome among the four phenotypes of polycystic ovary syndrome based on Rotterdam criteria. *Reprod Health*. 2015;12(7):1-9. DOI: 10.1186/1742-4755-12-7.
32. Yılmaz SA, Altınkaya SÖ, Kebabçılar A, *et al*. The relationship between Polycystic ovary syndrome and vitamin D levels. *Turk J Obstet Gynecol*. 2015;12(1):18-24. DOI: 10.4274/tjod.76148.
33. Wacker M, Holick MF. Sunlight and vitamin D: A global perspective for health. *Dermatoendocrinology*. 2013;5(1):51-08. https://doi.org/10.4161/derm.24494.
34. Tripathy S, Roy C, Banerjee A. Polycystic ovary syndrome: its genetics and treatments. *Indian J Physiol Allied Sci*. 2020;72(1):6-12. DOI: 10.55184/ijpas. v72i1.29.
35. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology*. 2000;141(4):1317-24. DOI: 10.1210/endo.141.4.7403.
36. Bolivar VC, Fontana BG, Fontana CG, Torres MM. Mechanisms involved in the relationship between vitamin D and insulin resistance: Impact on Clinical Practice. *Nutrients*. 2021;13(10):1-25. DOI: 10.3390/nu13103491.
37. Chakraborty S, Samaddar A, Das N, Saha SK, Basu BR. An insight of association of insulin resistance with polycystic ovary syndrome. *Indian J Clinical Anat Physiol*. 2021;8(4):248-54. DOI: 10.18231/j.ijcap.2021.055.
38. Yang M, Shen X, Lu D, Peng J, Zhou S, Xu L, Zhang J. Effects of vitamin D supplementation on ovulation and pregnancy in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Front Endocrinol (Lausanne)*. 2023;14:1-14. DOI: 10.3389/fendo.2023.1148556.

PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.