

ASSOCIATION OF VITAMIN-D WITH POLYCYSTIC OVARIAN SYNDROME: A CROSS-SECTIONAL STUDY

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is one of the common endocrine disorders in women of reproductive age group. Patients with PCOS often have vitamin D deficiency, which has been linked to a number of negative consequences in the course of the illness. However, it is still hard to draw a definite conclusion in the causal relationship due to inconsistent findings from various individual studies. The aim of the study is to determine the association of vitamin D level and polycystic ovarian syndrome (PCOS). **Materials and Methods:** This cross-sectional study was done in the Department of Biochemistry in collaboration with the Department of Obstetrics & Gynaecology, RIMS, Imphal, Manipur for a period of two years from October 2019 to September 2021. A total of 45 women aged 18 years and above with PCOS as cases and 45 age matched healthy women as controls were included in the study. Serum vitamin D level was measured using sandwich enzyme-linked immunosorbent assay (ELISA) and serum calcium was analysed using Randox autoanalyser. Spearman correlation coefficient was used to determine the association of Vitamin D levels with PCOS. The results were evaluated within 95% confidence interval (CI) and at a significance level of two-sided p-value less than 0.05. **Result:** Serum vitamin D level was much lower in cases (15.55 ± 5.6 ng/ml) compared to controls (74.45 ± 9.65 ng/ml) and the difference was statistically significant ($p < 0.05$). And serum calcium level in cases was 7.51 ± 0.63 mg% which was lower as compared to controls (9.52 ± 0.34 mg%) which was also found to be statistically significant (p value < 0.05). This study shows a positive correlation between vitamin D deficiency and number of PCOS cases. **Conclusion:** Our study found significant differences in the absolute level of serum vitamin D between PCOS patients and matched controls. Vitamin D deficiency is highly prevalent in PCOS women. Additionally, we find positive correlations between serum vitamin D deficiency and number of PCOS cases, suggesting the necessity to clarify the role of vitamin D in the pathogenesis of PCOS.

INTRODUCTION

PCOS, or polycystic ovarian syndrome, is a prevalent endocrine condition that affects around 18% of women in the reproductive age range.^[1] It has been estimated that between 9.13% and 36% of Indians suffer from PCOS.^[2] Anovulation, irregular

menstruation, infertility, and amenorrhea are its hallmarks.^[3] Type 2 diabetes mellitus, insulin resistance, central obesity, hypertension, impaired glucose tolerance, endometrial hyperplasia, and ovarian and endometrial cancer are among the several cardiovascular risk factors that are linked to it.^[4]

Vitamin D is essential for women's fertility and overall health.^[5] It can be acquired through diet or produced naturally by the skin when exposed to UV light. The skin contains 7-dehydrocholesterol, the precursor of vitamin D, which is subsequently hydroxylated in the kidney and liver. It first goes through photolysis to produce cholecalciferol, which is then hydroxylated at position 25 by the liver's 25-hydroxylase enzyme to produce 25-hydroxycholecalciferol. The active form of vitamin D, 1,25-dihydroxycholecalciferol, usually referred to as calcitriol, is then formed in the kidney by a second hydroxylation reaction that is mediated by the enzyme 1 α -hydroxylase.^[5,6] Vitamin D regulates calcium and bone homeostasis and is involved in a wide range of biological processes, including bone metabolism, immune system modulation, cell proliferation, and differentiation.^[7] The presence of vitamin D receptors (VDRs) in both human and animal granulosa cells and cumulus oophorus cells support the idea that vitamin D is important for the proper regulation of the female reproductive cycle.^[8] 1,25(OH)₂D₃, an active form of vitamin D, regulates the development and secretion of hormones, including human placental lactogen and human chorionic gonadotropin hormone; active vitamin D also regulates endometrial decidualization, and it is also a factor that may cause the invitro synthesis of progesterone, estradiol, and estrone.^[9,10] Additionally, it is required for females to produce estrogen efficiently.^[11] Despite being one of the greatest indicators of ovarian reserve, anti-mullerian hormone (AMH) expression and serum levels are known to be influenced by obesity and vitamin D levels.^[6]

Low levels of vitamin D have been linked to the development of obesity, insulin resistance, irregular menstruation and ovulation, hirsutism, hyperandrogenism, and cardiovascular disease.^[12] Studies have shown that 67-85% of women with polycystic ovarian syndrome (PCOS) are deficient in vitamin D.^[5,13,14] Also, new research indicates that vitamin D and calcium supplementation may help PCOS women become more insulin sensitive.^[15] Although some research has indicated a link between vitamin D levels and metabolic issues in PCOS, other studies found no connection between vitamin D and PCOS.^[16] The conflicting results from different studies make it difficult to get a firm judgment about the causal relationship. Furthermore, no research has been conducted in this region of the nation about vitamin D levels in PCOS cases. In light of this, the current study was carried out to assess vitamin D levels and their correlation with PCOS.

MATERIALS AND METHODS

This cross-sectional study consisted of a total of 45 women aged 18 years and above with PCOS as cases and 45 age matched healthy women as controls. Ethical approval of the study was obtained from the Research Ethics Board, Institutional Ethics

Committee (IEC), Regional Institute of Medical Sciences, Imphal. All patients had signed an informed consent.

Inclusion Criteria

- Diagnosed cases of PCOS based on Rotterdam criteria attending Gynaecology OPD irrespective of socio-economic status and ethnicity
- Apparently healthy females in reproductive age group that is 18-44 years

Exclusion Criteria

Subjects with the following conditions:

- Systemic diseases: chronic kidney disease, history of neoplastic disease
- Use of any medications that might affect the vitamin D level
- Smoking
- Chronic alcohol consumption
- Pregnancy

Blood sample collection and laboratory methods

5ml venous blood sample was collected in plain vial and serum was collected after centrifugation for 10 minutes at 2000-3000 rpm and stored at -20°C till analysis. Serum vitamin D level was measured using sandwich enzyme-linked immunosorbent assay (ELISA) and Serum Calcium was analysed using Randox Autoanalyser.

Statistical analysis: The data were analysed using IBM SPSS version 21.0 for windows. The results obtained were presented as mean \pm SD. Descriptive statistics like mean, percentage and proportion were used. Independent samples t-test, Chi-square test was used to compare means and Spearman's rank correlation was used to find out the correlation between the variables. p -value < 0.05 was taken as significant.

RESULTS

[Figure 1] shows that majority of the cases of PCOS occurred in the age groups of 18 to 25 years comprising of 22 (48%) of total cases, followed by 10 (23%) in the age group 26 to 30 years, 8 (18%) in the age group of 31-35 years and 5 (11%) in the age group of 36 -40 years.

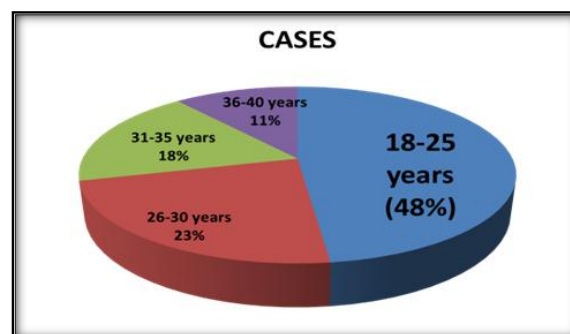


Figure 1: Pie- chart showing age distribution of the respondents among PCOS group

[Table 1] shows that there was no significant difference observed in age distribution between the

PCOS and non PCOS groups. However, BMI, WHR, SBP and DBP were significantly higher in PCOS group while Vitamin D and calcium levels were significantly lowered in PCOS group when compared with non-PCOS group.

[Table 2] shows that maximum number of cases ie. 38 (84.5%) had deficient serum vitamin D (< 20ng/ml). 5 cases (11.1%) had insufficient serum vitamin D (20-30 ng/ml) and only 2 cases (4.4%) had normal levels of serum vitamin D levels (30-100 ng/ml).

[Table 3] shows that vitamin D deficiency had significant positive correlation with PCOS ($r = +0.635$, $p < 0.001$).

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Table 1: Comparison of different biological and biochemical values between cases and controls.

| Parameters | Cases (PCOS) (Mean \pm SD) | Controls (Non-PCOS) (Mean \pm SD) | p-value |
|--------------------------|------------------------------|-------------------------------------|---------|
| Age (years) | 26.38 \pm 4.85 | 28.23 \pm 3.99 | 0.70 |
| BMI (kg/m ²) | 25.65 \pm 3.25 | 22.21 \pm 1.90 | < 0.001 |
| WHR | 0.87 \pm 0.04 | 0.78 \pm 0.03 | < 0.001 |
| SBP (mmHg) | 130.14 \pm 8.84 | 109.7 \pm 9.16 | < 0.001 |
| DBP (mmHg) | 94.22 \pm 4.99 | 72.88 \pm 5.48 | < 0.001 |
| Calcium (mg%) | 7.51 \pm 0.63 | 9.52 \pm 0.34 | < 0.001 |
| Vitamin D (ng/ml) | 15.55 \pm 5.6 | 74.45 \pm 9.65 | < 0.001 |

BMI-Body mass index, WHR-Waist hip ratio, SBP-Systolic blood pressure, DBP-Diastolic blood pressure

*Independent t- test

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Table 2: Distribution of PCOS group according to vitamin D levels

| Vitamin D level | Frequency | Percentage |
|-----------------------------|-----------|------------|
| Deficiency (<20 ng/ml) | 38 | 84.5% |
| Insufficiency (20-30 ng/ml) | 5 | 11.1% |
| Normal (30-100 ng/ml) | 2 | 4.4% |
| Total | 45 | 100% |

[Table 2] shows that maximum number of cases 38 (84.5%) had deficient serum vitamin D (< 20ng/ml). 5 cases (11.1%) had insufficient serum vitamin D (20-30 ng/ml) and only 2 cases (4.4%) had normal levels of serum vitamin D levels (30-100 ng/ml).

Table 3: Correlation between Vitamin D deficiency and number of PCOS.

| Spearman's correlation rho | Vitamin D deficiency | | Vitamin D deficiency | Cases of PCOS |
|----------------------------|----------------------|-------------------------|----------------------|---------------|
| | | Correlation coefficient | 1.000 | +0.635 |
| | | Sig(2 tailed) | - | <0.001 |
| | | N | 45 | 45 |

[Table 3] shows that vitamin D deficiency had significant positive correlation with PCOS($r = +0.635$, $p < 0.001$).

DISCUSSION

The study showed that the majority of PCOS occurred in the age groups of 18-25 years. This finding was similar to the study by Bashir R et al,^[17] who found out that the maximum prevalence of PCOS (i.e., 50%) was found in the age group of 15-24 years. It might be because PCOS is frequently identified in women in their early childbearing years, particularly when they experience oligomenorrhea and infertility and seek early medical attention for their symptoms.^[18] Research has shown that peripheral glucose consumption and low SHBG are positively correlated with insulin production. Low SHBG (Sex hormone binding globulin) levels may indicate hyperinsulinemia and insulin resistance, potentially identifying patients with PCOS.^[19]

The study showed that the level of serum Vitamin D levels was significantly lower in PCOS as compared to healthy subjects and out of 45 PCOS women, 38 (84.5%) were deficient in serum vitamin D with < 20ng/ml, 5 cases (11.1%) had insufficiency with serum vitamin D levels of 20-30ng/ml and only 2 cases (4.4%) had normal levels of serum vitamin D levels of 30-100ng/ml. These findings were similar to the results of Garg Get et al,^[20] who found that vitamin D deficiency (<20 ng/ml) was observed in 93.8% of PCOS subjects, while vitamin D insufficiency was observed in the remaining 6.2% of subjects. Gaikwad VA et al,^[21] in their study had also reported low levels of vitamin D in women with PCOS, which was comparable to our study. Vitamin D has significant roles in the reproductive system in addition to its impact in bone metabolism. Ovarian

and endometrial tissues have vitamin D receptors (VDRs), which are crucial for steroidogenesis and VDR gene polymorphisms which might be linked to PCOS and PCOS-related metabolic indicators. In addition, Dabrowski FA et al,^[22] study revealed that vitamin D modulates follicle stimulating hormone (FSH) sensitivity and AMH (anti-mullerian hormone) production patterns in ovarian granulosa cells, potentially contributing to the formation of ovarian follicles.

BMI and WHR were significantly higher in PCOS as compared to non PCOS subjects which was consistent with the findings of Wang Li et al.^[1] Thomson RL et al.^[5] reported that low 25(OH) vitamin-D levels were significantly affected by the degree of adiposity (BMI and WHR). As vitamin-D is fat soluble, a higher proportion of it is sequestered in adipose tissue, lowering its bioavailability in obese subjects.⁵ Alternatively obese subjects may spend less time outdoors exposed to sunlight that can lead to insufficient vitamin D biosynthesis in skin.

The present study showed that calcium level was significantly lowered in PCOS group when compared to non-PCOS group which was similar to the findings of Moini A et al.^[16] It has been suggested that calcium has a role in oocyte activation and maturation and hypothesized that disturbances in calcium homeostasis may mediate the pathogenesis of PCOS. The study showed positive correlation between the prevalence of vitamin D deficiency and number of cases of polycystic ovarian syndrome (PCOS). This finding was supported by the study conducted by Mogili KD et al,^[23] where vitamin D deficiency was observed in 80.3% women, 10.3% were vitamin D insufficient and only 9.4% were vitamin D sufficient. Vitamin D receptors (VDR) polymorphisms are thought to contribute to increase the risk for PCOS. In accordance with this theory, 1,25[OH]₂D controls several hundred genes, including some relevant to glucose homeostasis. Studies have linked VDR polymorphisms, such as iApa-I, Taq-I, Cdx2, Bsm-I and Fok-I to metabolic changes in PCOS. The rs757343 single nucleotide polymorphism was also associated with the severity of PCOS symptoms.^[24] SBP and DBP were significantly higher in PCOS than in non-PCOS subjects. Wu CH et al,^[24] reported that the incidence rates of hypertension were higher in the PCOS subjects as compared to control groups. A number of factors including insulin resistance (IR), hyperinsulinemia, hyperandrogenism, obesity, and cardiac autonomic dysfunction, are associated with the mechanism behind the higher prevalence of hypertension in PCOS. Insulin resistance (IR) causes compensatory hyperinsulinemia and vascular muscle wall hypertrophy, which further contributes to the development of hyperandrogenemia by interfering with endothelium-dependent vasodilatation processes. Endothelial dysfunction and high blood pressure are two potential consequences of PCOS's hyperandrogenic condition, which increases the risk of cardiovascular disorders. Through an imbalance in the autonomic nervous system, a decrease in nitric

oxide generation, and an increase in renal salt reabsorption, IR-related compensatory hyperinsulinemia may have an impact on blood pressure. The features of chronic comorbidities are comparable in persons with PCOS and hypertension. Compared to the control group, patients with PCOS are more likely to have diabetes mellitus (DM), hyperlipidemia, asthma, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and coronary artery disease (CAD).^[25] Some of the study's shortcomings were the fact that it was a single-centered cross-sectional study with a small sample size and no data on dietary vitamin D intake.

CONCLUSION

This study showed that serum vitamin D was significantly lower in cases of PCOS as compared to non-PCOS group. And there was positive correlation between the prevalence of vitamin D deficiency and number of PCOS cases. Thus, it can be concluded that serum vitamin D levels should be measured routinely in patients with PCOS and supplementation of it if required could be considered in the overall management of PCOS, together with weight loss and lifestyle modifications. However, further studies with larger population may be required to fully access the role of vitamin D in the prevention and management of women with PCOS.

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