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Original Article

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# Exploring the link between serum vitamin d and anti-müllerian hormone in polycystic ovary syndrome among reproductive-age women

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## Abstract

***Background & Aims*:** Several studies were conducted earlier to find the association between serum Vitamin- D and Anti-Müllerian hormone (AMH) levels among women with Polycystic Ovary Syndrome (PCOS). The results from the existing studies were inconsistent. The objective of the present study was to find out the association between serum Vitamin D and AMH levels among women with PCOS.

***Materials & Methods*:** A cross-sectional study was conducted in a Tertiary Care Teaching hospital in South India, 120 PCOS and 60 controls were included in the study. Age, Body Mass Index (BMI), Follicular Stimulating Hormone (FSH), Luteinizing hormone (LH), Estradiol (E2), Antral Follicular Count (AFC), Anti-Müllerian hormone (AMH), and 25-hydroxyvitamin D (25(OH)D) were analyzed among PCOS and control groups and comparisons were made.

***Results*:** There was no significant difference (*P =0.925*) in the Vitamin D levels among PCOS and control groups. There was a positive correlation (r = 0.392; *p = 0.002*) between Vitamin D levels and AMH levels among PCOS group.

***Conclusion*:** The present study revealed that there was no significant difference between Vitamin D levels among PCOS and control groups. There was weak positive correlation between Vitamin D levels and AMH levels among PCOS group. Multilinear regression analysis with AMH as dependent variable and other parameters as dependent variables in PCOS group revealed that AMH levels were independently correlated with age but not with Vitamin D.

***Keywords*:** Anti Müllerian Hormone, Polycystic Ovary Syndrome, Vitamin D Deficiency

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## Introduction

Vitamin D is a steroid hormone which regulates calcium and phosphate metabolism in the body. It can be synthesized in the skin from 7-dehydrocholesterol after ultraviolet exposure or be attained from diet such as oil-rich fish. Vitamin D3 (cholecalciferol) synthesized from the skin or attained from diet is biologically inactive. It's first hydroxylated in the liver

to 25-hydroxyvitamin D (25(OH)D) which is the main circulating form in the blood with a half-life of two to three weeks (1).

25(OH) D is also further converted to its active form, 1,25-dihydroxy vitamin D ($1,25(OH)_2D$) by the renal 25-hydroxyvitamin-D-1α-hydroxylase. $1,25(OH)_2D$ exerts its action in the target cells by binding to the vitamin D nuclear receptors.

The presence of Vitamin D receptor has been reported in the female reproductive system including ovaries, endometrium and uterus, suggesting that Vitamin D might have a part in the female reproductive process. The serum concentration of $1,25(OH)_2D$ is too low and the half-life is only 4 hours, making it a suboptimal marker of the Vitamin D status in the body (2).

One of the most prevalent reproductive endocrine disorders affecting women of reproductive age is Polycystic Ovarian Syndrome (PCOS). Ovulatory dysfunction, hyperandrogenism, and polycystic ovaries detected on ultrasonography are its defining characteristics. Additionally, it is typically linked to a higher risk of obesity, insulin resistance, and metabolic syndrome (2). With varying degrees of success, several researches have looked at the connection between PCOS's clinical symptoms and serum vitamin D levels. Although it has been previously stated that women with PCOS are more likely than non-PCOS women to be vitamin D deficient, additional research did not support this finding (3).

Anti-Müllerian hormone (AMH) is a hormone which, in adult women, is primarily produced by the small antralandpre-antral ovarian follicles. Higher levels of serum AMH has been observed in women with PCOS than in ovulatory healthy women (4). This is attributable both to the increased number of small antral follicles and to the increased AMH expression per granulosa cell in women with PCOS.

It's supposed that the excess of AMH in PCOS women decreases the sensitivity of antral follicles to FSH and hence results in follicular arrest. Androgens is thought to play a part in the early stages of folliculogenesis with an increasing number of growing follicles and proliferation of granulosa cells which will both beget an increase in AMH (5).

Since the presence of a functional vitamin D response element has been demonstrated in the promoter region of human AMH gene, there's considerable interest to explore the relationship between the two hormones (6).

In vitro, 25(OH) D levels in follicular fluid is negatively correlated with AMH and AMH receptor- II mRNA levels in cumulus granulosa cells of small follicles, suggesting that AMH signaling and steroidogenesis can be altered by vitamin D level (6).

Several studies were conducted to find the association between Vitamin D levels and serum AMH levels in PCOS subjects. Few studies (7,8) have reported that there was no correlation between Vitamin D level and AMH levels in PCOS while others (9, 10) have reported that there was a positive correlation between these two. Some other studies (11, 12) reported that there's a negative correlation between serum Vitamin D levels and AMH levels among women with PCOS. As the results from the former studies were inconsistent, we wanted to explore the link between serum Vitamin D and AMH levels in our study. The objective of the study was to explore the association of serum Vitamin D levels and AMH levels among women with PCOS.

## Materials & Methods

A cross-sectional study was conducted in a tertiary care teaching hospital in South India. Participants were recruited from the Gynecology and Internal Medicine Clinic of our hospital served between June 2022 and December 2022.

One hundred and twenty consecutive patients with a PCOS diagnosis based on the Rotterdam criteria (13), aged between 18 and 40 years and 60 age BMI matched controls were included in the study. We excluded patients with the history of thyroid disorders, hormone therapy, patients on calcium, vitamin D supplements and pregnancy. The Endocrine Society clinical practice guidelines define vitamin D deficiency as a ٢٥(OH)D<20 ng/mL, vitamin D insufficiency as a 25(OH)D between 21 and 29 ng/mL, and normal as a

25(OH)D of more than 30 ng/ml (14). PCOS group was divided into three sub groups: 25(OH)D-deficient, 25(OH)D-insufficient and normal ٢٥(OH)D based on the Endocrine Society clinical practice guidelines.

The present study was approved by the ethics committee of our institute No IEC/2022/2/48. Written informed consent was obtained from all study participants.

Age and Body Mass Index (BMI) were evaluated for all subjects. Weight was measured on an OMRON HN 286 digital scale (Omron Corporation, Kyoto, Japan with a sensitivity of 100 g), height on a SWWS05 stadiometer (Multicare Company, Delhi, India with a sensitivity of 0.1 cm), and Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Venous blood samples were collected from all subjects between days 3 and 6 of the menstrual cycle. The serum was separated from the whole blood and the necessary tests were carried out on the same day. FSH, LH, E2, AMH and vitamin D (total 25(OH)D) were measured on the Beckman Access2 immunoassay system using the chemiluminescence immunoassay method (CLIA) with commercial Beckman Coulter kits. On the same morning of the blood tests, a transvaginal ultrasound scan of antral follicle count (AFC) was performed using the model MINDRAY DC-7 T ultrasound machine in the lithotomy position. Antral follicles 2–10 mm in diameter were counted.

**Statistical Analysis:**

In the present study continuous variables were first evaluated using the Kolmogorov-Smirnov test to distinguish normality of distribution. Descriptive statistics were expressed as mean ± standard deviation (SD). Mean differences between study groups were compared by Student's t-test, and Mann-Whitney U-test was used to compare variables that were not normally distributed. Spearman's correlation was used to test any linear relationship between 25(OH)D levels and other study variables. Multivariate regression analysis of AMH as dependent variable was performed with other parameters in PCOS group. All the statistics were analyzed by SPSS version 21.

## Results

Comparison of age, BMI and other biochemical parameters among PCOS group and control group were represented in Table 1. There was no significant difference in the mean age, BMI levels among these two groups with P values 0.328 and 0.427 respectively. There was a significant ($P < 0.001$) difference between FSH, LH, E2 and AMH levels among PCOS and control groups.

25(OH)D levels in PCOS group and control group were 14.08±5.56 ng/ml and 13.96±5.40 ng/ml respectively in with P value of 0.925. There was no significant difference between Vitamin D levels among PCOS and control groups.

**Table 1:** Comparison of age, BMI, and Biochemical parameters between PCOS and Control group

| Parameter | PCOS group (n=120) | | Control group (n=60) | |
|---|---|---|---|---|
| | r value | p value | r value | p value |
| Age (years) | -0.295 | 0.022 | -0.278 | 0.137 |
| BMI | -0.142 | 0.278 | 0.086 | 0.652 |
| AMH (ng/ml) | 0.392 | 0.002 | 0.326 | 0.078 |
| FSH (mlU/mL) | 0.083 | 0.531 | 0.256 | 0.172 |
| LH (mlU/mL) | -0.182 | 0.164 | 0.193 | 0.307 |
| E2 (pg/ml) | -0.125 | 0.341 | 0.069 | 0.716 |
| AFC | 0.105 | 0.423 | -4.30 | 0.018 |

Correlation of Vitamin D levels with various parameters in the PCOS and Control Groups was presented in Table 2**.** There was a positive correlation (r=0.392; *p=0.002*) between Vitamin D levels and AMH levels among PCOS group. Even though P value is 0.002, r value is 0.392 indicating there was no strong association. No significant correlation was found between Vitamin D values and age, BMI, FSH, LH, E2, AFC values among PCOS group. Our study did not find any correlation between Vitamin D levels and age, BMI, AMH, FSH, LH, E2, AFC levels among the control group.

**Table 2:** Correlation of Vitamin D levels with various parameters in the PCOS and Control Groups

| Parameter | PCOS Group (n=120) | Control Group (n=60) | P value |
|---|---|---|---|
| Age (years) | 31.98 ± 4.62 | 33.07± 5.48 | 0.328 |
| BMI | 25.24± 1.19 | 25.44± 0.84 | 0.427 |
| AMH (ng/ml) | 5.13± 1.47 | 2.88± 1.77 | <0.001 |
| FSH (mlU/mL) | 4.57± 2.11 | 13.52 ± 9.65 | <0.001 |
| LH (mlU/mL) | 3.03± 2.21 | 7.44± 2.76 | <0.001 |
| E2 (pg/ml) | 17.86± 5.6 | 48.04± 21.05 | <0.001 |
| AFC | 11.96± 8.43 | 12.20± 0.88 | 0.227 |
| 25-OHD (ng/ml) | 14.08±5.56 | 13.96± 5.40 | 0.925 |

Multilinear regression analysis with AMH as dependent variable and other parameters as dependent variables in PCOS group revealed that AMH levels were independently correlated with age (t = -5.612 and *p <0.001*). All other parameters including Vitamin D levels did not show any independent correlation (Table 3).

**Table 3:** Multiple linear regression analysis with AMH as dependent variable

| Coefficients[a] | | | | | |
|---|---|---|---|---|---|
| Model | Unstandardized Coefficients | | Standardized Coefficientst | | Sig. |
| | B | Std. Error | Beta | | |
| (Constant) | 14.248 | 4.007 | | 3.556 | .001 |
| Age | -.195 | .035 | -.610 | -5.612 | .000 |
| BMI | -.108 | .126 | -.088 | -.855 | .397 |
| FSH | -.018 | .086 | -.026 | -.208 | .836 |
| LH | -.013 | .075 | -.019 | -.170 | .865 |
| E2 | -.012 | .030 | -.046 | -.395 | .695 |
| AFC | -.046 | .192 | -.027 | -.242 | .810 |
| Vit D | .052 | .028 | .196 | 1.835 | .072 |

a. Dependent Variable: AMH

## Discussion

In the present study, we have tried to establish a link if any, between Vitamin D status and serum AMH levels among women with PCOS of reproductive age group. Existing literature on the relation between Vitamin D status and PCOS was inconsistent. A cross-sectional study conducted by Merhi et al. (14)showed serum Vitamin D levels were positively correlated with serum AMH levels in late reproductive age (> 40 years) and weak negative correlation between serum Vitamin D

levels and serum AMH levels among young (<35 years) individuals. A study by Arameshet al. (9) reported that serum Vitamin D levels were significantly positively correlated with AMH levels. Another study conducted by Wong et al. (10)also found that Vitamin D levels were positively correlated with AMH levels. In the present study, we found a weak positive correlation between serum Vitamin D levels and AMH levels among PCOS group, our study results are consistent with these studies.

Studies conducted by Kim S et al. (7) and Jukic AMZ et al. (8), and Chang EM et al. (15) showed there was no correlation between serum Vitamin D levels and AMH levels. But Other studies conducted by Bednarska-Czerwinska A et al. (11) and Liu X et al. (12) reported there was a negative correlation between serum Vitamin D levels and AMH levels.

Variations in the findings of cross-sectional studies mentioned above may be due to heterogeneity in the study population that can contribute to conflicting data reported by these studies. Moreover, Vitamin D levels are influenced by race, ethnicity, geographical area, exposure to sun light and have been suggested to play a role in ovarian reserve difference among the different populations. Another factor affecting the results is the range of Vitamin D levels in a given population varies widely between different studies.

Another limitation of cross-sectional studies is their nature of evaluating a single point which doesn't take into consideration of individual variations in AMH and Vitamin D levels as well as seasonal variations in Vitamin D levels.

Interventional studied conducted to find the association between Vitamin D and AMH levels found that there was an increase in AMH levels in non-PCOS Vitamin D deficient women following both short term (16) and long term (17, 18) Vitamin D supplementations. But Cappy et al. (19) found that there was no change in the serum AMH levels following Vitamin D supplementation in either PCOS or non PCOS women.

Other interventional studies by Irani M et al. (20) and Dastorani M et al. (21) showed Vitamin D supplementation led to a decrease in AMH levels only in women with PCOS. Women with PCOS usually have abnormally high serum AMH levels which are reflective of the quantity of their numerous arrested small ovarian antral follicles. The increase in the serum AMH levels correlates with the severity of PCOS manifestations like amenorrhea and hyperandrogenism(22).

Szafarowska et al. (23) reported that polymorphisms Fok1 (rs228570) and Apa1 (rs7975232) in the Vitamin D receptor (VDR) gene are associated with elevated AMH levels in PCOS. These genetic variations can contribute to differences in the findings on the association between serum Vitamin D levels and serum AMH levels among PCOS subjects.

## Conclusion

The present study revealed that there was no significant difference between Vitamin D levels among PCOS and control groups. There was weak positive correlation between Vitamin D levels and AMH levels among PCOS group. Multilinear regression analysis with AMH as dependent variable and other parameters as dependent variables in PCOS group revealed that AMH levels were independently correlated with age but not with Vitamin D. Large randomized control trials of Vitamin D supplementations among different Vitamin D statuses are necessary to understand the complex nature of the relationship between serum Vitamin D levels and AMH levels among PCOS subjects.

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## Conflict of interest

The authors have no conflict of interest in this study.

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No declared.

## Data Availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

## Author's Contribution

(Write in short form: e.g. VKA: Performed work, VKA and RRK: Designed and generated idea, VKA: prepared manuscript, VKA and RRK: statistics)

## Ethical Statement

The present study was approved by the ethics committee of our institute No IEC/2022/2/48. Written informed consent was obtained from all study participants.

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