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Testosterone and Vitamin D Levels in Polycystic Ovary Syndrome Patients: A Case-Control Study

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Abstract

Introduction:

Polycystic ovary syndrome(PCOS) is a prevalent endocrine disorder characterized by hormonal imbalances, including elevated testosterone levels. Vitamin D, a hormone with diverse physiological functions, has been implicated in PCOS pathogenesis and management. This study aims to investigate the relationship between testosterone levels and vitamin D status in PCOS patients compared to healthy controls.

Methods:

100 PCOS patients and 100 age-matched healthy women were recruited for this study. PCOS patients were diagnosed based on Rotterdam criteria. Serum testosterone levels were measured using immunoassay, while vitamin D status by chemiluminescent immunoassay. Anthropometric measurements, including body mass index(BMI), were recorded. Statistical analyses, like t-tests and logistic regression, done to evaluate the association between testosterone levels, vitamin D status and PCOS.

Results:

Testosterone levels was significantly increased and vitamin D levels decreased in PCOS when compared to control. Logistic regression analysis also revealed same report after adjusting for age and BMI.

Conclusion:

In this study higher testosterone levels align with the characteristic hyperandrogenism in PCOS, while the lower vitamin D status may play a role in the pathogenesis of PCOS. Further research is warranted to elucidate the underlying mechanisms linking testosterone and vitamin D in PCOS and to explore the therapeutic benefits of vitamin D supplementation in PCOS patients.

Keywords : *Polycystic Ovary Syndrome, Testosterone, Vitamin D, Body mass index, Hyperandrogenism*

Introduction :

Polycystic Ovary Syndrome (PCOS) is a hormonal disorder commonly found in women of childbearing age. It's characterized by certain features like multiple cysts on the ovaries, higher levels of male hormones (hyperandrogenism), and difficulties with ovulation. While the exact cause of PCOS isn't fully understood, it's believed to involve various factors, including genetic predisposition. One key aspect in the development of PCOS is insulin resistance, which means the body's cells don't respond properly to insulin, leading to higher levels of insulin in the blood.

This insulin resistance often occurs alongside weight gain, particularly around the waist, and is closely linked to higher levels of male hormones and problems with ovarian function. When obesity and insulin resistance are present, they can worsen the symptoms of hyperandrogenism, contributing to the hormonal imbalances seen in PCOS.(2),(3) Additionally, women with PCOS are at a higher risk of developing certain health conditions like cardiovascular disease, type 2 diabetes, high blood pressure, endometrial cancer, and inflammatory conditions. This increased risk is often associated with higher levels of fat tissue and elevated male hormones in the body.

In essence, PCOS is a complex condition involving hormonal imbalances, insulin resistance, and other metabolic factors. These factors can lead to a range of symptoms and increase the risk of developing serious health problems. Understanding and managing PCOS typically involves addressing these underlying hormonal and metabolic issues through lifestyle changes, medications, and sometimes assisted reproductive technologies to help with fertility issues.(1)

Materials & Methods :

The study aimed to investigate the relationship between polycystic ovary syndrome (PCOS), serum testosterone levels, and vitamin D status among a cohort of 100 PCOS patients and 100 age-matched healthy women. PCOS diagnosis was established using the Rotterdam criteria, a widely accepted diagnostic framework encompassing clinical, hormonal, and ultrasound findings.

In this research endeavor, the recruitment process was meticulous, involving the selection of both PCOS patients and healthy controls to ensure a representative sample. Age matching between the PCOS group and healthy controls was crucial to minimize potential confounding variables related to age-related hormonal changes. By enrolling age-matched healthy women, the study aimed to discern specific associations between PCOS and the variables of interest while controlling for age-related effects.

Serum testosterone levels, a hallmark feature in PCOS, were assessed using immunoassay techniques, which provide precise measurements of circulating testosterone concentrations. This methodology ensures the accuracy and reliability of testosterone assessments, crucial for elucidating its role in PCOS pathophysiology. Furthermore, the use of immunoassay techniques allows for standardized comparisons across study participants, facilitating robust statistical analyses.

Similarly, vitamin D status was evaluated using chemiluminescent immunoassay, a sensitive and specific method for quantifying serum vitamin D levels. Given the emerging evidence implicating vitamin D deficiency in PCOS development and progression, investigating its association with PCOS becomes imperative. By employing chemiluminescent immunoassay, the study aimed to delineate potential links between vitamin D status and PCOS, shedding light on its potential therapeutic implications.

Anthropometric measurements, including body mass index (BMI), were meticulously recorded to characterize the study participants' adiposity profiles. Obesity and insulin resistance are common features of PCOS, exerting significant impacts on its pathogenesis and clinical manifestations. Therefore, assessing BMI provides valuable insights into the metabolic milieu of PCOS patients and its potential interplay with testosterone levels and vitamin D status.

Statistical analyses constituted a pivotal component of this study, enabling the elucidation of associations between testosterone levels, vitamin D status, and PCOS. T-tests were employed to compare mean testosterone levels and vitamin D concentrations between PCOS patients and healthy controls, elucidating potential differences between the two groups. Logistic regression analysis was utilized to assess the independent contributions of testosterone levels and vitamin D status to PCOS risk, accounting for potential confounders such as BMI and age.

By employing a rigorous statistical framework, the study aimed to discern the nuanced relationships between testosterone levels, vitamin D status, and PCOS while controlling for potential confounding factors. This analytical approach enhances the validity and generalizability of study findings, facilitating meaningful interpretations and implications for clinical practice and future research endeavors.

Overall, this study represents a comprehensive investigation into the intricate interplay between PCOS, serum testosterone levels, and vitamin D status. By leveraging robust methodologies and rigorous statistical analyses, it advances our understanding of PCOS pathophysiology and highlights potential avenues for therapeutic interventions and preventative strategies.

Result :

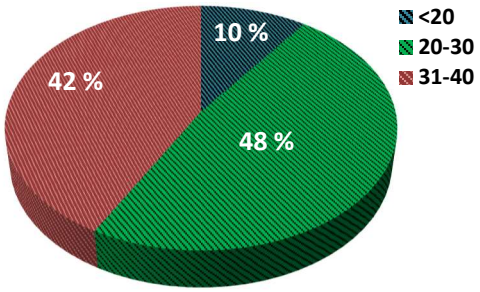
Study Design:

A Comparative case control study to find the relationship between testosterone levels and vitamin D status in PCOS patients compared to healthy controls, To find the relationship between testosterone levels and BMI in PCOS patients compared to healthy controls. To find the relationship between BMI and vitamin D status in PCOS patients compared to healthy controls

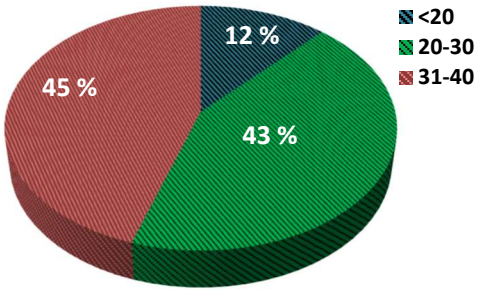
Table 1: Age in years –Frequency distribution in two groups of patients studied

Age in Years	PCOS	NORMAL	Total
<20	10(10%)	12(12%)	22(11%)
20-30	48(48%)	43(43%)	91(45.5%)
31-40	42(42%)	45(45%)	87(43.5%)
Total	100(100%)	100(100%)	200(100%)

P=0.755, Not Significant, Chi-Square Test



Age in Years : PCOS



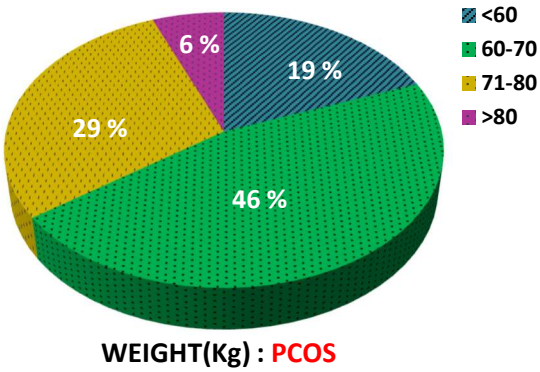
Age in Years : NORMAL

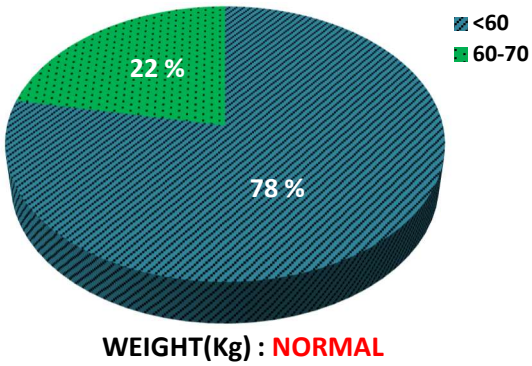
This table represents the frequency distribution of patients studied, categorized by age group, in two groups: PCOS patients and normal (non-PCOS) individuals.
The "PCOS" group contains the count of PCOS patients aged less than 20 years, which is 10 individuals, constituting 10% of the total PCOS group. Similarly, at "<20" age group and the "NORMAL" group contains the count of normal individuals aged less than 20 years, which is 12 individuals, constituting 12% of the total normal group.
Inbetween "20-30" age group, there are 48 PCOS patients (48%) and 43 normal individuals (43%), indicating a similar distribution between the two groups within this age range .Similarly, in the "31-40" age group, there are 42 PCOS patients (42%) and 45 normal individuals (45%), again showcasing a comparable distribution between PCOS and normal groups in this age range.
Overall, this frequency distribution table offers a succinct representation of the age distribution patterns among PCOS patients and normal individuals, enabling insights into potential age-related differences or similarities between the two groups within the studied population.

Table 2: WEIGHT (Kg)- Frequency distribution in two groups of patients studied

WEIGHT(Kg)	PCOS	NORMAL	Total
<60	19(19%)	78(78%)	97(48.5%)
60-70	46(46%)	22(22%)	68(34%)
71-80	29(29%)	0(0%)	29(14.5%)
>80	6(6%)	0(0%)	6(3%)
Total	100(100%)	100(100%)	200(100%)

P≤0.001**, Significant, Fisher Exact Test



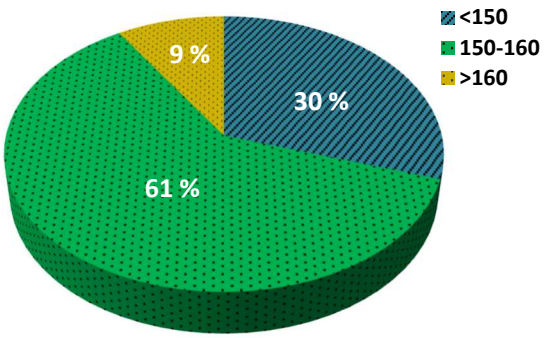


This table displays the frequency distribution of patients studied, categorized by weight (in kilograms), across two groups: PCOS patients and normal (non-PCOS) individuals. The data is presented in tabular form, with weight categories defined as "<60", "60-70", "71-80", and ">80" kilograms, and frequencies provided for both PCOS and normal groups, along with their respective percentages and totals.
This table allows for a visual comparison of weight distribution between PCOS patients and normal individuals within each weight category. For instance, in the "<60" kg weight category, there are 19 PCOS patients (19%) and 78 normal individuals (78%), indicating a notable difference in weight distribution between the two groups in this weight range. Conversely, in the "60-70" kg weight category, there are 46 PCOS patients (46%) and 22 normal individuals (22%), showing a higher proportion of PCOS patients within this weight range compared to normal individuals.
Overall, this frequency distribution table provides valuable insights into the weight distribution patterns among PCOS patients and normal individuals, highlighting potential differences or similarities in weight profiles between the two groups within the studied population.

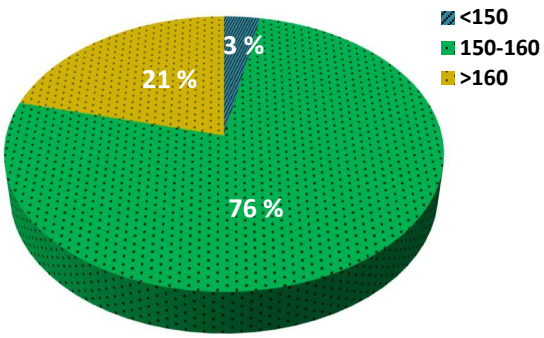
Table 3: HEIGHT (Cm)- Frequency distribution in two groups of patients studied

HEIGHT(Cm)	PCOS	NORMAL	Total
<150	30(30%)	3(3%)	33(16.5%)
150-160	61(61%)	76(76%)	137(68.5%)
>160	9(9%)	21(21%)	30(15%)
Total	100(100%)	100(100%)	200(100%)

P≤0.001**, Significant, Fisher Exact Test



HEIGHT(Cm) : PCOS



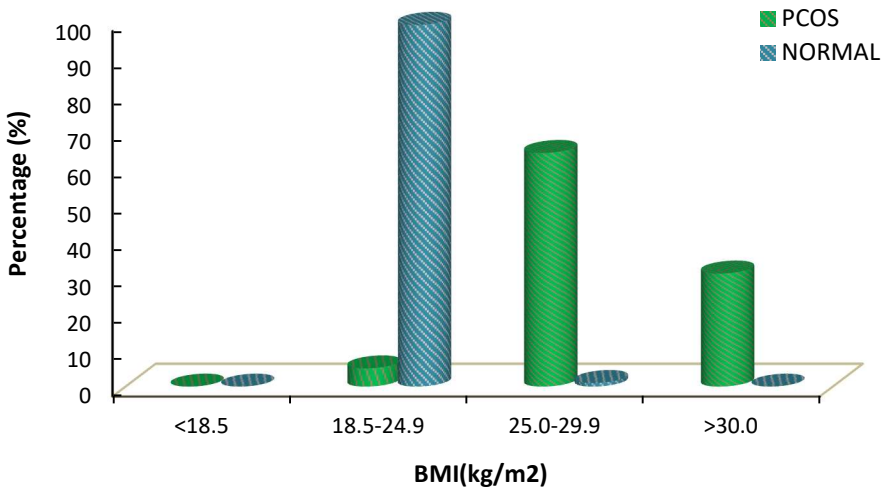
HEIGHT(Cm) : NORMAL

This table compares the height distribution between individuals with Polycystic Ovary Syndrome (PCOS) and those without. In the PCOS group, 30% are under 150 cm, 61% are between 150-160 cm, and 9% are over 160 cm. Conversely, in the normal group, 3% are under 150 cm, 76% are between 150-160 cm, and 21% are over 160 cm. Overall, PCOS individuals exhibit a higher proportion of shorter stature.

Table 4: BMI(kg/m2)- Frequency distribution in two groups of patients studied

BMI(kg/m2)	PCOS	NORMAL	Total
<18.5	0(0%)	0(0%)	0(0%)
18.5-24.9	5(5%)	99(99%)	104(52%)
25.0-29.9	64(64%)	1(1%)	65(32.5%)
>30.0	31(31%)	0(0%)	31(15.5%)
Total	100(100%)	100(100%)	200(100%)

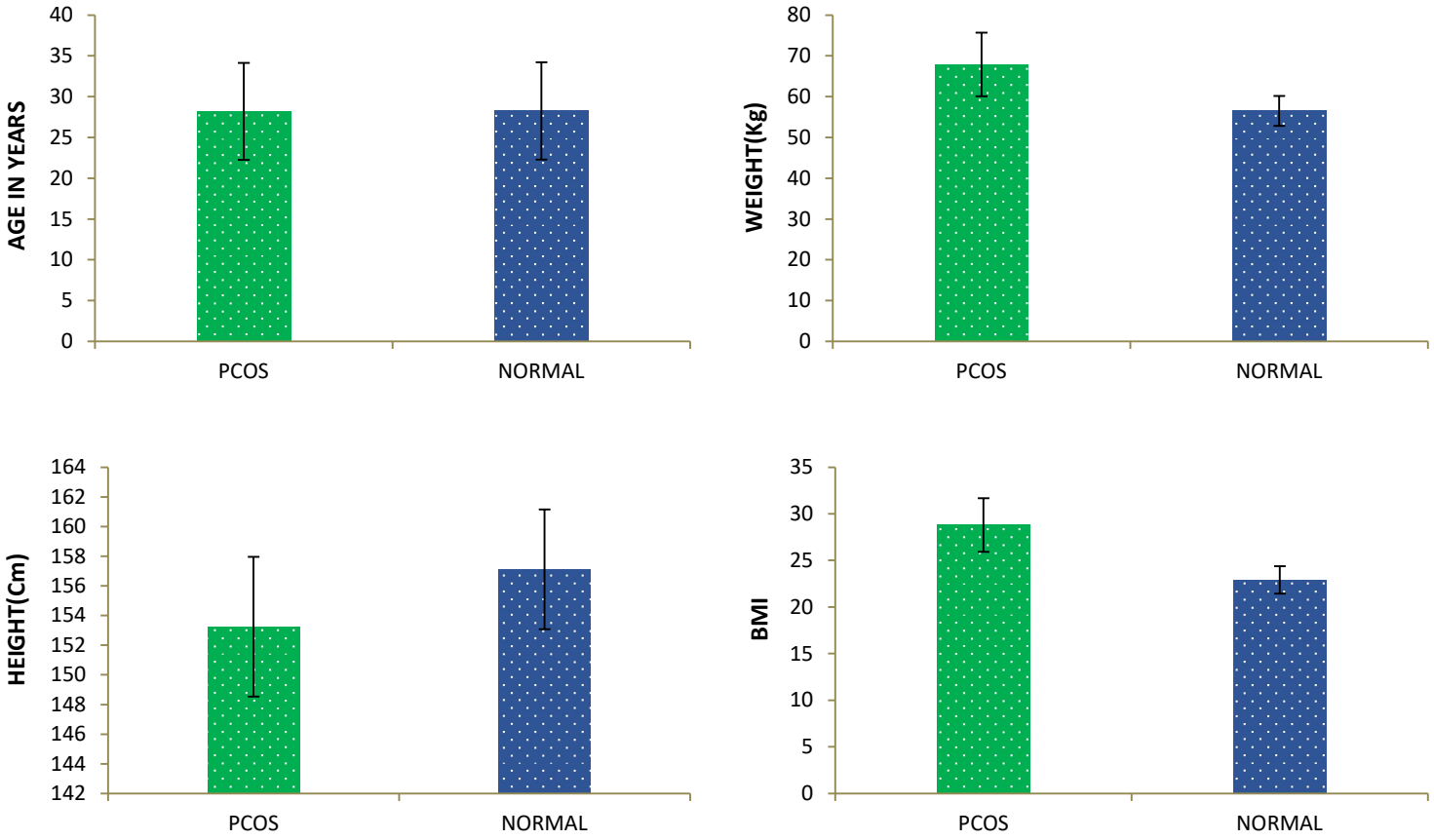
P≤0.001**, Significant, Fisher Exact Test



This table presents the distribution of Body Mass Index (BMI) categories among individuals with Polycystic Ovary Syndrome (PCOS) compared to those without. In the PCOS group, 64% fall into the overweight category (25.0-29.9 kg/m²), and 31% are classified as obese (>30.0 kg/m²). Conversely, the normal group predominantly comprises individuals with a healthy BMI (18.5-24.9 kg/m²). No individuals with PCOS have a BMI below 18.5 kg/m².

Table 5:Comparison of baseline variables in two groups of patients studied

Variables	PCOS	NORMAL	Total	P Value
AGE IN YEARS	28.2±5.93	28.25±5.96	28.23±5.93	0.953
WEIGHT(Kg)	67.92±7.79	56.51±3.69	62.21±8.34	<0.001**
HEIGHT(Cm)	153.26±4.71	157.12±4.03	155.19±4.78	<0.001**
BMI	28.8±2.87	22.91±1.47	25.86±3.73	<0.001**

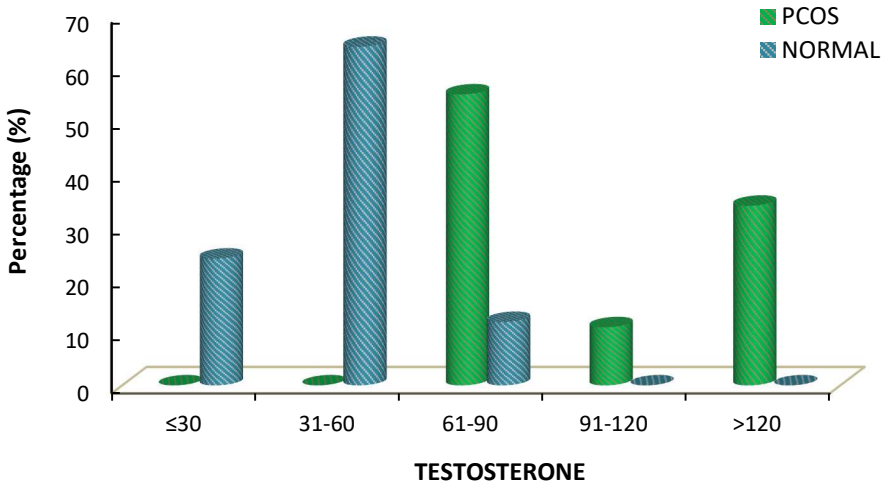


This table compares baseline variables between patients with Polycystic Ovary Syndrome (PCOS) and those without (NORMAL). The mean age is similar between groups (PCOS: 28.2 years, NORMAL: 28.25 years, p=0.953). However, significant differences are observed in weight (PCOS: 67.92 kg, NORMAL: 56.51 kg, p<0.001), height (PCOS: 153.26 cm, NORMAL: 157.12 cm, p<0.001), and BMI (PCOS: 28.8 kg/m², NORMAL: 22.91 kg/m², p<0.001).

Table 6: TESTOSTERONE- Frequency distribution in two groups of patients studied

TESTOSTERONE	PCOS	NORMAL	Total
≤30	0(0%)	24(24%)	24(12%)
31-60	0(0%)	64(64%)	64(32%)
61-90	55(55%)	12(12%)	67(33.5%)
91-120	11(11%)	0(0%)	11(5.5%)
>120	34(34%)	0(0%)	34(17%)
Total	100(100%)	100(100%)	200(100%)

P≤0.001**, Significant, Fisher Exact Test

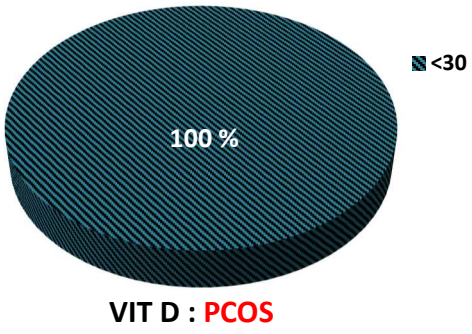


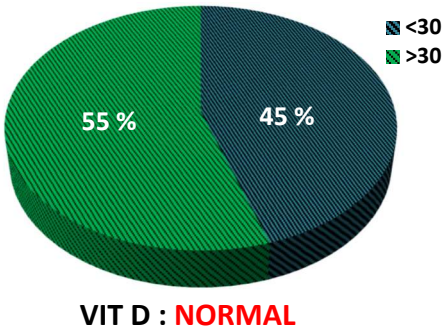
This table illustrates the frequency distribution of testosterone levels in patients with Polycystic Ovary Syndrome (PCOS) compared to those without (NORMAL). In the PCOS group, the majority have testosterone levels between 61-90 ng/dL (55%), followed by levels >120 ng/dL (34%). Conversely, in the NORMAL group, most have levels between 31-60 ng/dL (64%). Testosterone levels in the PCOS group exhibit a broader range and higher values compared to the NORMAL group.

Table 7: VIT D- Frequency distribution in two groups of patients studied

VIT D	PCOS	NORMAL	Total
<30	100(100%)	45(45%)	145(72.5%)
>30	0(0%)	55(55%)	55(27.5%)
Total	100(100%)	100(100%)	200(100%)

P≤0.001**, Significant, Fisher Exact Test

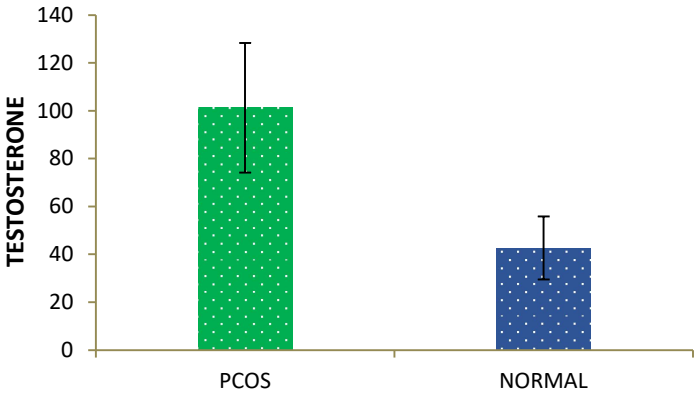


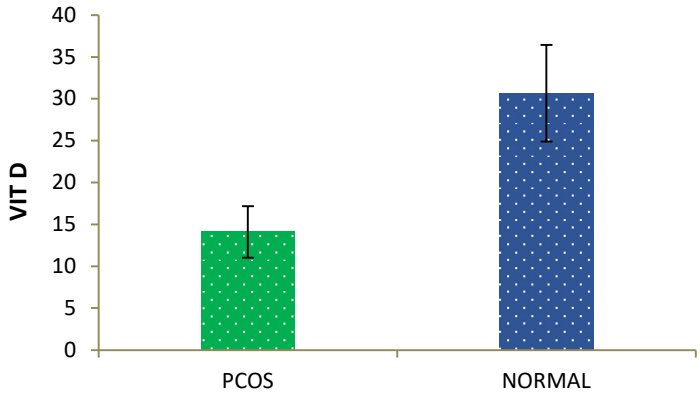


This table displays the frequency distribution of Vitamin D levels in patients with Polycystic Ovary Syndrome (PCOS) versus those without (NORMAL). In the PCOS group, all patients have Vitamin D levels below 30 ng/mL, while in the NORMAL group, all patients have levels above 30 ng/mL. Consequently, there's a stark contrast in Vitamin D status between the two groups, highlighting a potential association between PCOS and Vitamin D deficiency.

Table 8:Comparison of outcome variables in two groups of patients studied

Variables	PCOS	NORMAL	Total	P Value
TESTOSTERONE	101.32±27.09	42.66±13.17	71.99±36.27	<0.001**
VIT D	14.12±3.08	30.69±5.77	22.41±9.5	<0.001**





This table compares outcome variables between patients with Polycystic Ovary Syndrome (PCOS) and those without (NORMAL). Significant differences are observed in both testosterone levels (PCOS: 101.32 ng/dL, NORMAL: 42.66 ng/dL, p<0.001) and Vitamin D levels (PCOS: 14.12 ng/mL, NORMAL:

30.69 ng/mL, p<0.001). PCOS patients exhibit higher testosterone levels and lower Vitamin D levels compared to the NORMAL group.

Table 9a: PEARSON CORRELATION

TESTOSTERONE VS	r value	p Value
AGE IN YEARS	0.266	<0.001**
WEIGHT(Kg)	0.662	<0.001**
HEIGHT(Cm)	-0.276	<0.001**
BMI	0.719	<0.001**
VIT D	-0.683	<0.001**

Table 9b: PEARSON CORRELATION

VIT D VS	r value	p Value
AGE IN YEARS	0.046	0.520
WEIGHT(Kg)	-0.571	<0.001**
HEIGHT(Cm)	0.298	<0.001**
BMI	-0.646	<0.001**
TESTOSTERONE	-0.683	<0.001**

In Table 9a, correlations between testosterone levels and various parameters are examined. Testosterone positively correlates with weight ($r = 0.662$, $p < 0.001$) and BMI ($r = 0.719$, $p < 0.001$) but negatively with height ($r = -0.276$, $p < 0.001$) and Vitamin D levels ($r = -0.683$, $p < 0.001$).
In Table 9b, correlations between Vitamin D levels and parameters are explored. Vitamin D negatively correlates with weight ($r = -0.571$, $p < 0.001$), BMI ($r = -0.646$, $p < 0.001$), and testosterone ($r = -0.683$, $p < 0.001$). However, it positively correlates with height ($r = 0.298$, $p < 0.001$).
These correlations suggest intricate relationships between hormonal status, anthropometric measures, and Vitamin D levels, highlighting potential clinical implications in conditions like PCOS.

Discussion:
According to the study conducted by Hind et al, IVF/ICSI outcomes between 69 PCOS and 69 non-PCOS women, focusing on BMI's impact. Higher BMI negatively affected key fertility outcomes in both groups, with PCOS women showing more pronounced challenges. Categorically, BMI ≥ 30 kg/m² correlated with significantly lower pregnancy rates, especially in the PCOS group(4)
Yali et al found the fact that, Other studies have found that testosterone levels in the follicular fluid of PCOS patients are higher compared to those in non-PCOS controls.(5,6)

According to the recent studies conducted Vitamin D deficiency is frequently observed in the general population, but it's particularly prevalent among individuals with polycystic ovary syndrome (PCOS). PCOS is characterized by insulin resistance, a condition closely associated with Vitamin D levels.(7,8,9) Recent research indicates that continuous Vitamin D supplementation at doses below 4000 IU per day can enhance glucose metabolism and reduce insulin resistance. Additionally, it appears to have positive effects on menstrual regularity and the reduction of hyperandrogenism in individuals with PCOS. A study conducted by Zeeshan et al. compared the effects of Vitamin D supplementation against a placebo group and found that it led to improvements in alanine aminotransferase levels and insulin resistance. Specifically, in obese Vitamin D-deficient women with PCOS, Vitamin D supplementation resulted in modest improvements in insulin resistance characteristics.(10-14)

According to the studies conducted by Feyzi Gokosmanoglu et al, found that Group 1, which had lower vitamin D levels, exhibited higher serum testosterone, DHEA-S, and LH levels compared to the group with normal vitamin D levels ($p<0.05$). This suggests that vitamin D deficiency is associated with elevated androgen hormone levels in individuals with PCOS. Therefore, vitamin D levels could be used as an indicator of metabolic and hormonal status in PCOS patients. Additionally, other studies have shown that vitamin D replacement therapy can improve insulin sensitivity and reduce androgen levels in PCOS patients who are deficient in vitamin D.(15)
Studies conducted by Parikh et alshows that in the human ovarian tissue, the production of estrogen and progesterone is stimulated by 1,25(OH)2D3 (active vitamin D), while testosterone production decreases. This reduction in testosterone may occur due to the enhancement of aromatase activity by vitamin D(15)
Thomas at al study shows Vitamin D deficiency leads to calcium imbalance, which contributes to follicular arrest in women with PCOS, causing menstrual irregularities and fertility issues(16-18)
Pal et al. conducted a before-and-after study involving 12 obese women aged 19 to 39 years who have polycystic ovary syndrome (PCOS). They discovered that vitamin D supplementation can reduce the levels of total testosterone and androstenedione in these women(19)
Wahr et al. (2011) observed similar results, where 23 out of 46 patients aged 18–46 with irregular menstruation experienced regular menstrual cycles after 24 weeks of vitamin D supplementation.(20,21)

Conclusion-

This study found that elevated testosterone levels are consistent with the typical hyperandrogenism seen in PCOS, suggesting that low vitamin D status might contribute to the condition's development. The findings highlight the need for further research to understand the mechanisms that link testosterone and vitamin D in PCOS. Additionally, exploring the therapeutic benefits of vitamin D supplementation in PCOS patients could provide valuable insights for treatment. This research could help clarify how vitamin D influences hormonal balance and the potential for improving PCOS symptoms through vitamin D supplementation.

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