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Research Paper

Vitamin D and Carboxy-terminal Cross-linking Telopeptide of Type-1 Collagen in Polycystic Ovary Syndrome and Their Relation with Hyperandrogenism

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ABSTRACT:

BACKGROUND:

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Key features such as hyperandrogenism, hyperinsulinemia, and obesity can affect bone mineral density and biochemical markers of bone turnover. Vitamin D is linked to reproductive health, metabolic changes, and mental well-being in PCOS patients.

OBJECTIVE:

To assess the relationship between vitamin D, CTX (C-Telopeptide of type 1 collagen), and testosterone levels in women with PCOS.

PATIENTS AND METHODS:

This comparative cross-sectional study included 78 women with PCOS and 82 healthy controls. Serum testosterone and vitamin D levels were measured using a COBAS® e411 analyzer, while CTX levels were quantified using an ELISA sandwich kit.

RESULTS:

Women with PCOS had significantly higher weight, BMI, waist circumference, and waist-to-hip ratio compared to controls. Vitamin D levels were significantly lower in the PCOS group, though there was no correlation with serum testosterone or CTX levels. However, women with deficient vitamin D showed significantly higher CTX and testosterone levels compared to those with sufficient or insufficient vitamin D. Both CTX and testosterone were notably elevated in women with PCOS compared to healthy controls.

CONCLUSION:

Vitamin D levels are lower in Iraqi women with PCOS, highlighting the importance of vitamin D supplementation in their treatment. Elevated CTX levels suggest a higher risk for bone disease in PCOS patients.

KEYWORDS: Polycystic ovary syndrome, vitamin D, bone mineral density

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INTRODUCTION:

Polycystic ovary syndrome (PCOS), also called "hyperandrogenic anovulation" or "Stein–Leventhal syndrome", results from combination of many factors, when it is a polygenic condition that commonly affects women of reproductive age. Key characteristics of PCOS, such as hyperandrogenism, hyperinsulinemia, and obesity [(1)], affects bone mineral density (BMD) and the bone turnover biochemical markers, offering a noninvasive way to assess bone health [(2)].

Diagnosis of PCOS usually depends on history, examination, and investigations (laboratory, serum androgen level and pelvic ultrasound) [(3).] Although biochemical markers associated with PCOS include increased LH secretion, normal or slightly low FSH, and an elevated LH/FSH ratio (markers of ovarian secretion). Elevated testosterone and increased dehydroepiandrosterone sulfate (DHEAS)- reflecting adrenal secretion- were also found in patients with PCOS and may affect bone turnover and BMD [(4)].

Vitamin D3 is a prohormone produced in the skin through ultraviolet irradiation, playing a vital role in regulating the metabolism of calcium and

mineralization process of bone. It has 2 forms: ergocalciferol (VD2) and cholecalciferol (VD3), with VD3 synthesized endogenously in the body [5]. The Endocrine Society of North America classifies 25(OH)D levels in the serum as deficient, when the level is less than 20 ng/mL, insufficient when the level lies between 20-29 ng/mL, or sufficient when the level is 30 ng/mL or more [6,7]. Research shows an inversely proportional correlation among serum Vitamin D levels and PCOS's metabolic and hormonal disturbances, suggesting that low Vitamin D levels may worsen symptoms [8].

The marker of bone resorption, C-terminal telopeptide (CTX) or carboxy-terminal collagen crosslinks is used to measure the rate of bone turnover. Elevated CTX levels have been observed in PCOS women and may influence the ALP levels of bone and the overall BMD. Screening for CTX levels in PCOS patients could affect overall bone health [9,10].

Understanding how Vitamin D, CTX, and hyperandrogenism relate can shed light on how PCOS affects bone health. This emphasizes the importance of a comprehensive approach to treatment that considers both hormone levels and bone health. Ultimately, this research could lead to better management strategies that improve hormonal balance, metabolic health, and long-term bone health for women with PCOS, enhancing their overall quality of life.

PATIENTS AND METHODS:

Seventy-eight Iraqi women between 20-40 years old, who had polycystic ovarian syndrome (PCOS), were recruited for this comparative cross-sectional study, they were chosen by simple random sampling. These patients were seen at gynecological private clinics and Al-Basrah Hospital for Women and Children between February 1, 2022, and December 1, 2022. Rotterdam criteria were used to diagnose PCOS [11], which necessitates the presence of at least 2 of three conditions: irregular menstrual cycles, clinical signs, or biochemical changes of hyperandrogenism, and the presence of polycystic ovaries on ultrasound.

For comparison, 82 healthy, fertile women with regular menstrual cycles and normal ultrasound findings were selected as the control group. These women, who had no signs of hyperandrogenism, were attending the hospital and clinics as companions and were matched with the PCOS group for age.

Women who were diagnosed with PCOS during their first visit were included. **Exclusion criteria were**: 1) Using hormonal medications within the previous three months, 2) Inherited or acquired bone disorders. any endocrine disorders, such as thyroid or parathyroid disease, diabetes mellitus, Cushing syndrome, or congenital adrenal hyperplasia. 3) Chronic diseases including renal, liver, heart disease, hypertension, or bronchial asthma.

After confirming the diagnostic criteria, a detailed medical history was taken using a structured questionnaire. Data collected included the patient's name, age, and anthropometric measures (weight, height, body mass index, waist circumference, and hip circumference).

After an eight-hour fasting, 5 mL of blood was drawn from a peripheral vein at the time of diagnosis, before any treatment was initiated. The blood was allowed to clot for 30 minutes and then centrifuged at ambient temperature for 20 minutes. On the same day, 3 mL of the extracted serum was used to measure testosterone and vitamin D levels using the COBAS® e411 analyzer. The remaining 2 mL of serum was aliquoted, labelled, and stored at -80°C for future analysis of CTX levels.

The competition principle was used for measuring testosterone and vitamin D; it involves competitive binding assays where a known amount of enzyme-labeled hormone competes with the hormone in the sample for binding sites on specific antibodies. In testosterone assays, the sample and labeled hormone are added to wells coated with anti-testosterone antibodies; higher testosterone levels in the sample result in less binding of the labeled hormone, which inversely correlates with signal intensity. Similarly, vitamin D measurements follow this principle, competing for binding to antibodies specific to vitamin D, allowing for quantification based on the resulting signal.

ELISA based on biotin double antibody sandwich technology to measure CTX-1. Wells pre-coated with monoclonal antibodies for CTX-1 are filled with the sample, followed by incubation. Anti-CTX-1 antibodies labelled with biotin are added, forming an immune complex with streptavidin-HRP. After washing to remove unbound enzymes, the substrate was introduced, resulting in a colour change from blue to yellow with acid. The solution's colour intensity correlates positively with CTX-1 concentration.

Verbal consent from the patients, as well as Basrah Health directorate approval, was obtained before

data collection. The researchers followed the ethics principles stated in the Declaration of Helsinki for research ethics.

For the purpose of statistical analysis, the Statistical Package for Social Sciences (SPSS), version 25 was used.

RESULTS:

Table (1) presents a comparison of various health parameters between control and patient (PCOS) groups. Notably, the patient group shows significantly higher anthropometric measures and total serum testosterone levels, when compared to the control group, with p-values indicate high statistical significance ($p < 0.001$). Conversely, the patients group has a significantly lower vitamin D level. Age and height do not exhibit significant differences between the groups, as indicated by the higher p-values.

Table 1: Comparison of demographic, anthropometric, testosterone, CTX, and vitamin D levels in patients with controls.

Parameter	Control (N=82)	Patient (N=78)	P-value
Age (Year)	27.32 ± 4.81	27.03 ± 5.01	0.804
Weight (kg)	65.70 ± 4.10	73.85 ± 7.57	0.0001*
Height (cm)	161.89 ± 3.92	160.55 ± 2.65	0.19
BMI	25.05 ± 1.41	28.64 ± 2.77	0.0001*
Waist (cm)	82.87 ± 3.55	89.50 ± 5.10	0.0001*
Hip (cm)	104.79 ± 3.33	104.69 ± 3.13	0.764
Waist/Hip Ratio (W/H)	0.79 ± 0.03	0.86 ± 0.04	0.0001*
S. Total Testosterone (ng/ml)	0.25 ± 0.09	0.37 ± 0.11	0.0001*
CTX (ng/ml)	0.45 ± 0.13	0.57 ± 0.11	0.0001*
Vit. D (ng/ml)	23.74 ± 5.47	17.26 ± 5.39	0.0001**

*** Mann-Whitney U**

**** Independent t-test**

Table (2) illustrates the distribution of Vitamin D levels among control and patient groups, revealing a significant difference ($p = 0.0001$) in deficiency rates. A striking 74.4% of patients are classified as Vitamin D deficient, compared to only 29.3% of controls. Insufficient levels are also more prevalent in patients (23.1%) compared to controls (57.3%), while sufficient levels are minimal in both groups, particularly in patients (2.6%).

Table 2: Comparison between patient and control groups categorized according to vitamin D level.

		Group		Total	P-value*
		Control	Patient		
Vitamin D level categories	**Deficient**	24	58	82	0.0001
		29.3%	74.4%	51.3%	
	Insufficient	47	18	65	
		57.3%	23.1%	40.6%	
	Sufficient	11	2	13	
		13.4%	2.6%	8.1%	
Total		82	78	160	
		100.0%	100.0%	100.0%	

***Chi-Square Test**

Table (3) summarizes the correlations between Vitamin D, serum testosterone, and CTX in the patient group. Notably, there is a directly proportional correlation between Vitamin D levels and serum testosterone levels ($r = 0.001$, $p = 0.990$), indicating a potential relationship, albeit not statistically significant. Conversely, the correlation between serum testosterone and CTX is negative ($r = -0.205$) with a p-value of 0.071, suggesting a borderline significant inverse relationship. Vitamin D shows a negligible inverse correlation with CTX ($r = -0.042$, $p = 0.712$), indicating no significant association.

Table 3: Spearman's nonparametric correlations of testosterone, CTX, and vitamin D levels in patients.

Parameter	Correlation with Vitamin D (ng/ml)	P-value	Correlation with S. Testosterone (ng/ml)	P-value	Correlation with CTX (ng/ml)	P-value
Vitamin D (ng/ml)	N/A	N/A	0.001	0.990	-0.042	0.712
S. Testosterone (ng/ml)	0.001	0.990	N/A	N/A	-0.205	0.071
CTX (ng/ml)	-0.042	0.712	-0.205	0.071	N/A	N/A

DISCUSSION:

Weight, BMI, waist, and waist-to-hip ratio were significantly higher in patients (P value <0. 05) than in controls. The findings were in agreement with Bhattacharya K et al. which reported an increased in Weight, BMI, and W/H ratio in patients with PCOS because PCOS prevalence is increasing in overweight or obese women due to decreased insulin resistance [12]. In contrast, Šumarac-Dumanović M et al. stated that there was no any difference in weight, BMI, or W/H ratio between PCOS patients and controls because not all patients with PCOS have basal and stimulated hyperinsulinemia and insulin resistance. In PCOS patients, about 40-70% suffer from insulin resistance and 30-50% suffer from obesity [13].

There is a statistically significant difference between the two compared groups (p-value <0. 05) regarding serum testosterone level. In Abdelazim et al., study the serum total testosterone was significantly higher among women with PCOS compared to healthy controls which agrees with the current study result. Because total and free testosterone are the main increased androgens and can be used to diagnose excess ovarian androgens [14].

Our result agrees with Nagy E. E. and colleagues who found that serum C-terminal telopeptide (CTX) was significantly higher among women with PCOS compared to healthy controls so they have a higher association with fracture risk and osteoporosis [15]. Similarly, Afaf Zia et al. found increased CTX levels in PCOS patients. PCOS is an inflammatory condition, that affects bone metabolism and bone turnover markers [9]. In contrast, Lingaiah S et al. found that there were no significant differences in CTX between patients and controls, because the relationships between hormones and bone turnover markers may differ before and after peak bone mass [16].

In the present study, vitamin D was significantly lower in women with PCOS compared to healthy controls (P value < 0. 05). In agreement with the current study, studies conducted by Elida et al. [17], Morgante G et al. [18], A Mohan et al. [19], which reported a decrease in serum Vitamin D in patients with PCOS more than controls because obesity is often associated with hypovitaminosis D due to increasing storage of 25(OH)D in adipose tissue, which is common problem in PCOS patients.

Among the PCOS group, the finding in this study revealed that serum total testosterone was not correlated with other variables which include CTX (P=0. 71, R = -0. 205), and Vitamin D (P =0. 990, R =0. 001). Our result agrees with Mesinovic J et al. which found no association between vitamin D and androgens in PCOS patients because many genetic and environmental factors regulate vitamin D receptors, so these polymorphisms may explain the finding [20].

In our study, we observed a weak negative correlation between CTX and serum levels of Vitamin D. This aligns with findings from an observational study indicating that young women with PCOS exhibited decreased bone formation markers, while CTX levels showed no significant difference when compared to healthy women (9).

CONCLUSION:

That patients with polycystic ovary syndrome (PCOS) had significantly lower serum levels of Vitamin D compared to healthy female controls. Additionally, the findings revealed significantly higher levels of CTX and total testosterone in patients with PCOS, indicating an elevated risk of metabolic bone disease.

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