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THE ASSOCIATION OF SERUM VITAMIN D AND SUPEROXIDE DISMUTASE LEVEL IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Abstract: Objective: This study aimed at investigating the association of serum vitamin D and superoxide dismutase level in women with polycystic ovary syndrome (PCOS) as well as non-PCOS healthy ovulatory women. Methods: A total of 20 patients with polycystic ovaries and 20 of their age matched healthy control participates was included in this study. The patient samples were collected from women who reproductive age between 18 and 40 with PCOS. Results: The results of the current study showed a high level of SOD enzyme concentration versus decrease in the level of vitamin D in the PCOS patients compared to the healthy group. Conclusion: There is an opposite effect of superoxide dismutase level and vitamin D on polycystic ovary syndrome (PCOS).

Keywords: Polycystic Ovary Syndrome (PCOS), Serum Vitamin D, Superoxide Dismutase (SOD), Oxidative stress in PCOS, Vitamin D deficiency in PCOS

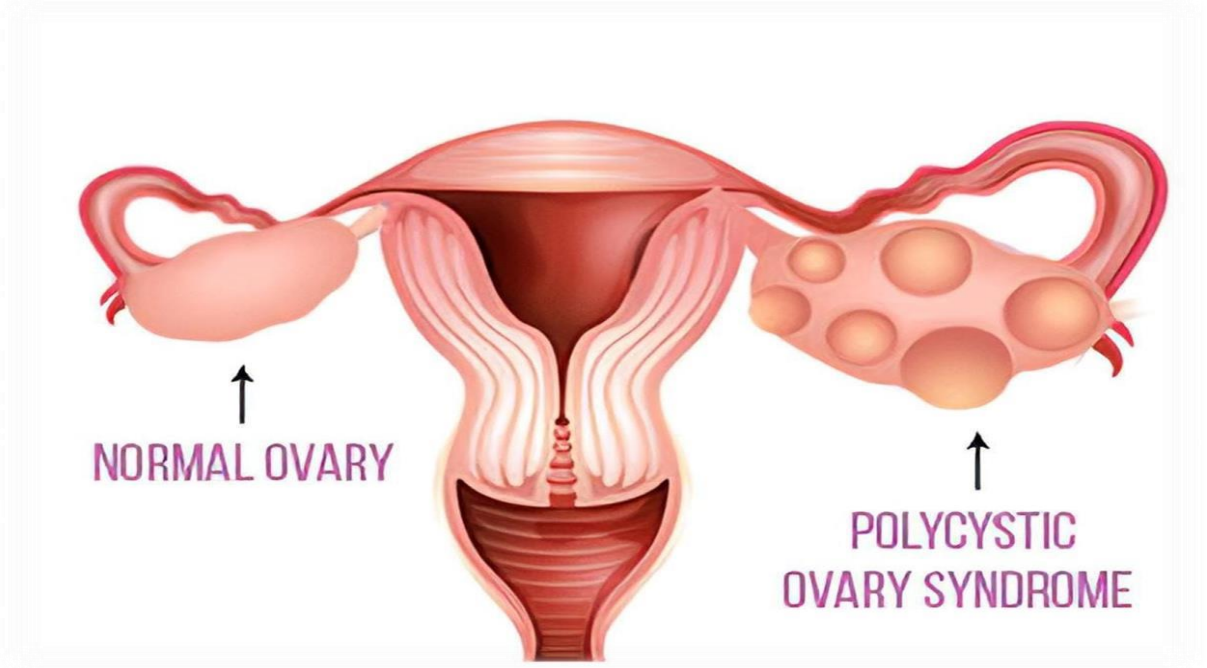
Introduction

Polycystic ovary/ovarian syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among reproductive-aged women, characterized by hormonal imbalances and the presence of multiple cysts on the ovaries. The exact causes of PCOS are not fully understood, but it is believed to involve a combination of genetic, environmental, and hormonal factor (1).

Polycystic Ovaries between 5% and 26% of women are affected by PCOS, depending on the Adolescence. diagnostic criteria applied (2,3). Diagnosing PCOS in adolescents is difficult because PCOS and puberty have similar features. These include irregular menstrual cycles and acne. For an accurate diagnosis, adolescents should have all three elements of the Rotterdam criteria for PCOS. Hyperandrogenemia is the main marker for PCOS in adolescents (4). PCOS have three to four times the rate of pregnancy-induced hypertension and preeclampsia (5). There is also a significantly increased risk of endometrial cancer in women with PCOS.25 Late reproductive to menopausal age In addition to endometrial cancer, women over 54 years of age with PCOS were found to have a significant risk of ovarian cancer, though the risk for breast cancer is not significantly increased by having PCOS (6).

Older women with PCOS have a fourfold to sixfold increase of diabetes compared with women without PCOS.7 Older women with PCOS also have more severe hirsutism, in addition on increased number of metabolic and cardiovascular risk factors (7).



The effect of superoxide dismutase on polycystic ovaries

Superoxide dismutase (SOD) is an enzyme that plays a critical role in protecting cells from the toxic effects of reactive oxygen species (ROS). SOD is an antioxidant enzyme that plays a crucial role in protecting cells from oxidative damage. Oxidative stress, which occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of the body's antioxidant defenses to neutralize them, has been implicated in the pathogenesis of several diseases, including polycystic ovary syndrome (PCOS) (8).

These ROS are generated as by-products of normal cellular metabolism and can cause damage to DNA, proteins, and other cellular components if they are not eliminated or neutralized. SOD catalyzes the dismutation of superoxide radicals (O2-) into molecular oxygen (O2) and hydrogen peroxide (H2O2). The enzyme is found in nearly all living organisms, and several types of SOD exist, including copper/zinc SOD (Cu/Zn SOD), manganese SOD (Mn SOD), and extracellular SOD (EC SOD) (9).

There have been several studies investigating the potential role of SOD in the treatment of PCOS. One study published in the Journal of Assisted Reproduction and Genetics found that treatment with SOD resulted in a significant reduction in the number of ovarian cysts and an improvement in menstrual regularity in women with PCOS. Another study published in the International Journal of Fertility and Sterility found that SOD levels were significantly lower in women with PCOS compared to healthy controls, and that SOD levels were negatively correlated with markers of oxidative stress and inflammation (10).

The effect of vitamin-D- on polycystic ovaries

Vitamin D is a fat-soluble vitamin that is essential for maintaining strong bones and teeth, as well as a healthy immune system. It is also important for regulating the levels of calcium and phosphorus in the body (11).

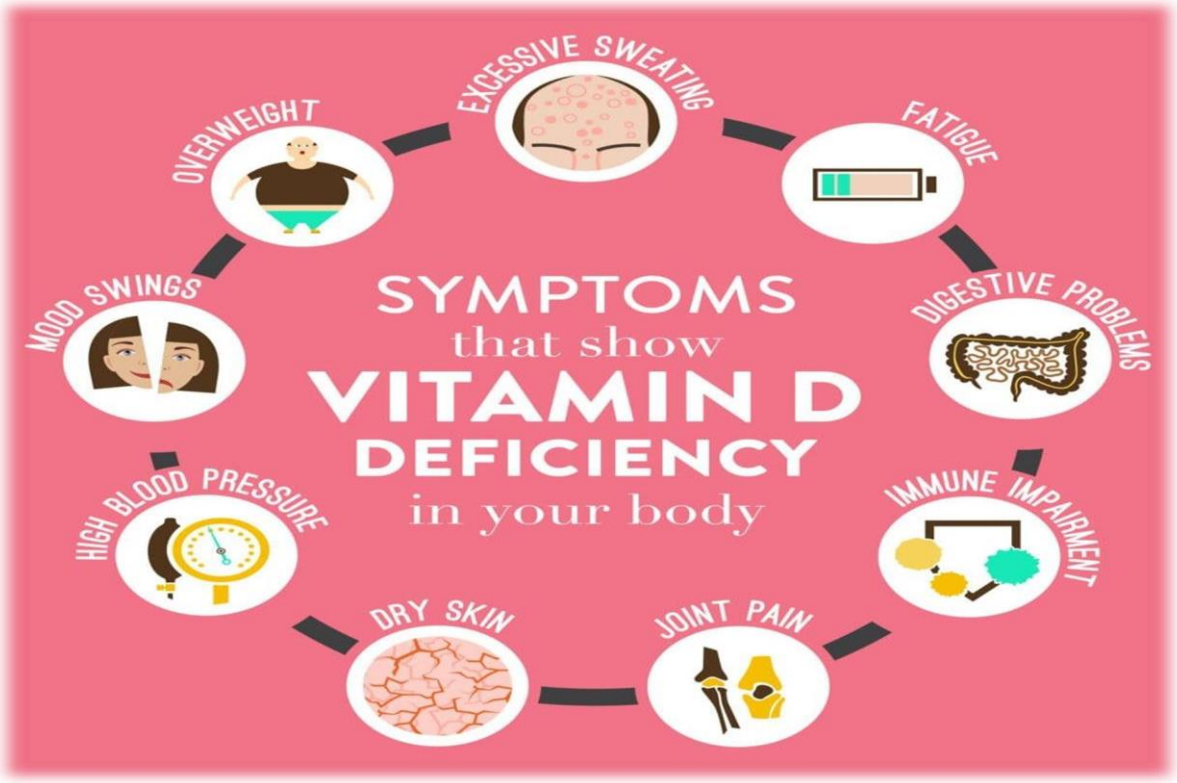
The body can produce vitamin D when the skin is exposed to sunlight, specifically UVB radiation. However, some people may not get enough vitamin D through sunlight alone, especially those who live in areas with limited sunlight or who spend most of their time indoors. In these cases, it may be necessary to get vitamin D through dietary sources or supplements.

The two main forms of vitamin D are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is found in some plant-based foods, while vitamin D3 is produced in the skin when it is exposed to sunlight. Both forms can also be obtained through supplements and fortified foods such as milk (12).

Vitamin D deficiency is a common problem, particularly in areas with limited sunlight exposure, and can lead to conditions such as rickets in children and osteoporosis in adults. Some studies have also suggested that vitamin D deficiency may be associated with an increased risk of certain types of cancer, autoimmune diseases, and infection (13).

There is some evidence to suggest that vitamin D may play a role in the development of PCOS. Research studies have found that women with PCOS are more likely to have vitamin D deficiency than those without the condition. Additionally, low vitamin D levels have been associated with insulin resistance, a common feature of PCOS (14).

While the exact relationship between PCOS and vitamin D is not yet fully understood, it is possible that vitamin D supplementation may help improve some symptoms of PCOS. However, more research is needed to determine the optimal dose and duration of supplementation, as well as its long-term effects on PCOS and overall health. It is always advisable to consult with a healthcare provider before starting any new supplements or making significant changes to your diet (15).



Methods

Equipment

All common laboratory chemicals and devices which applicated in this. study are of analytical. r grade, and they are received from the following companies as shown in Table, (2-1, 2-2):

Equipment	Company	Origin
Electronic Sensitive balance	Sartorius	Germany
Water bath	Memmert	Germany
Centrifuge	Hettich	Germany
Incubator	Memmert	Germany
Length scale	Salter	England
Micropipettes	Hettich	Germany
Spectrophotometer	Apel(Pd303uv)	Germany
Refrigerator (deep freeze)	National	Japan

Chemicals

Table (2-2): Chemical used in the study

Chemicals	Company	Origin
Dipotassium Hydrogen Orthophosphate . Trihydrate ($K_2HPO_4.3H_2O$)	**BDH**	**England**
Ethylenediaminetetraacetic acid disodium (EDTA-2Na)	**BDH**	**England**
Potassium dihydrogen Orthophosphate .Trihydrate ($K_2HPO_4.3H2O$)	**BDH**	**England**
Nitro blue tetrazolium (NBT-2HCl)	**Sigma**	**USA**
Triton x-100	**Fluka**	**Switzerland**
Riboflavin	**BDH**	**England**
Potassium Cyanide(KCN)	**BDH**	**England**

Kits

Table (2-3): Kit used in the study are following

Kit	Supplier Company
Serum vitamin D	Integrated Sciences (AUSTRALIA)

Sampling

A total of 20 patients with polycystic ovaries and 20 of their age matched healthy control participates was included in this study. The patient samples were collected from women who reproductive age between 18 and 40 with PCOS. Patients' family history, age, and body mass index (BMI) in (kg/m2) were determined using a specific questionnaire. After the clinical evaluation, patients with additional disorders and pregnancy were excluded from the assessment. From each participating subject (patient and control), five to ten milliliters of blood were collected in. a plain gel tube and placed for around (10 min at (25°C) to clot, and tubes were separated, by. centrifuge at. (3000, rpm). for, (10 min) to, separate serum. . The serum was divided into two Eppendorf tubes and stored, in deep, Freeze (-20. °C).

Measurement of Body Mass Index (BMI)

Measurement of the, body. mass, index is dependent on the height and weight of the person. It is a simple weight (kg) divided by height (m^2) and is used primarily to classify adult underweight, normal, overweight and obesity, as shown in equation (1)

$$\text{Weight (Kg) BMI} = \frac{}{\text{Height (m)}^2} \quad \ldots (1)$$

Determination of Total Serum Superoxide Dismutase (SOD) Activity

In the last decade, different methods were used to estimate the most important antioxidant enzyme SOD activity. Some methods used expensive commercial kit or enzymatic reaction or such as using cytochrome C (16) and xanthine oxidase (17). These methods are expensive and very protein sensitive which susceptible to denaturation. Nitro blue tetrazolium was also used where the sample was radiated using a straight neon lamp 20 watt (18).

The principle

The activity of SOD has determined in serum by indirect method of Beyer which deepened on the indirect method (riboflavin/NBT method) (18). This method was based on the principle that

superoxide radicals cause the reduction of nitro blue tetrazolium (NBT) to violet formazan, that can be measured at 470560 nm spectrophotometrically (19). As shown in figure (2-1).

Reagents:

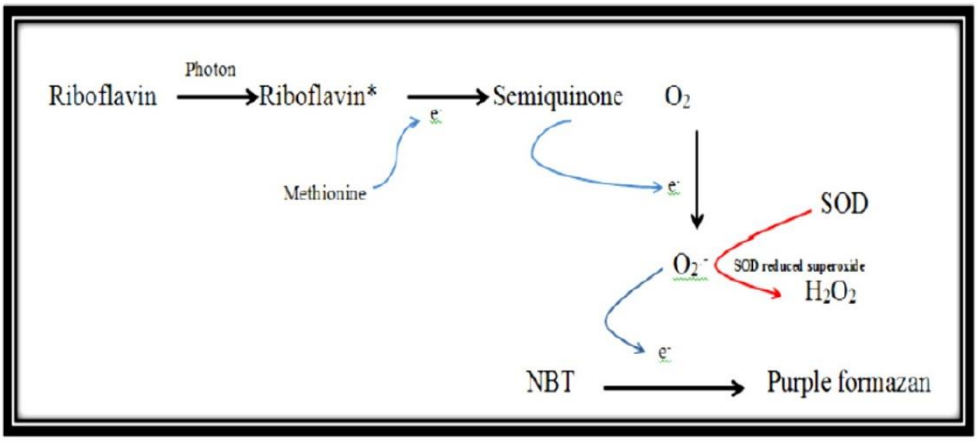


Figure 2-8: Indirect method (riboflavin/NBT) for measuring superoxide dismutase activity .Illumination of riboflavin in the presence of O_2 and electron donor like methionine generates superoxide anions and this anions can change NBT to formazan, but SOD inhibits this reaction. (15).

Reagents Preparation

Reagents	Preparation
R1	Solution (A): Weights of 2.9942 g of $K_2HPO_4.3H_2O$ and 0.0098 g of EDTA-2Na were dissolved in a little amount of deionized water and diluted up to 100 mL with deionized water. Solution (B): Weights of 1.7854 g of KH_2PO_4 and 0.0098 g of EDTA-2Na were dissolved in a little amount of deionized water, and the volume was made up to 100 mL with deionized water. **Working phosphate buffer solution was prepared as following:** 50ml of solution (A) was mixed with a volume of solution (B) until the pH adjusted to (7.8) .
R2	Weights of 0.0044g of riboflavin dissolved in a little amount of deionized water and then the volume was made up to 100 mL with deionized water

The working solution was prepared as following:

a:

Component	Concentration W/V[a]	Volume (mL)	Final Concentration
Phosphate buffer (pH 7.8)		11.875	103 mM
L-Methionine	300 mg/10mL	1.5	19.8 mM
NBT-2HCl[b]	14.1 mg/10 mL	1	0.114 mM
Triton x-100	100 mg/10 mL	0.75	$4.96x10^{-2}$ (W/V)
Total		15.125	

Solutions were prepared in deionized water

b: This solution was stored in a brown bottle in a refrigerator

Procedure*:*

□□□Aliquots of 0.5 mL of the working mixture solutions were diluted with 0.4 mL of deionized water.

□□□ A volume of 10 µL of serum in a covered tube was well mixed. A blank tube was running in parallel, in which serum was replaced by 10µL buffer solution. □□□ To each, tube (10µL) of R2 was immediately added and mixed. The absorbance was immediately assessed at 560 nm.

4.All tubes were illuminated in an aluminum foil box with two 20-Watt fluorescent lamps at 25oC for 7 min. Tubes were extracted after 7 min and the absorbance was immediately assessed at 560 nm.

Calculations

From the following formula the inhibition percentage of SOD activity was determined:

$$\text{SOD activity (inhibition \%)} = \frac{(AB2-AB1)-(AS2-AS1)}{(AB2-AB1)} \times 100 \quad \ldots (2)$$

Where:

AS1 = The absorbance of sample before illumination.

AS2 = The absorbance of sample after illumination.

AB1 = The absorbance of blank before illumination.

AB2 = The absorbance of blank after illumination.

One .unit of, .SOD is the enzyme activity that inhibits 50% of NBT formazan formation (20, 21) .Therefore, the SOD activity in the assay of riboflavin/NBT in unit (U/mL) is expressed according to the following equations:

50% inhibition =1 Unit of SOD = Y

Y unit= X% * 1/50

Y unit is from =10µL of serum contain enzyme

Total volume of serum contain enzyme = 10µL/1000 (1 ml =1000 µL)

(Y/10) *1000 = SOD activity (unit /ml)

Determination" of vitamin D:

Precision of the VIDAS® 25-OH Vitamin D Total Assay was determined across the dynamic

range using assay controls and serum samples according to CLSI protocol EP9-A2. Two replicates of each sample were tested twice per day in separate runs, for 5 days, using 3 reagent lots on 2 different VIDAS® systems. Assay precision was determined using samples with 25(OH)D ranging from about 10 to 130 ng/mL. Limit of blank, limit of detection, and functional sensitivity. The limit of blank (LoB) and the limit of detection (LoD) are determined according to CLSI protocol EP17-A2. Limit of Quantification (LoQ) -or functional sensitivity- corresponds to the lowest amount of 25(OH)D that can be quantitatively determined with stated accuracy of CV.

Assay Methodology:

The VIDAS® 25-OH Vitamin D Total Assay design is based on a 2-step competitive immunoassay. First step: serum or plasma 25(OH)D is dissociated from its protein carrier (DBP) then added to alkaline phosphatase (ALP) conjugated Vitamin D-specific antibody. Second step: unbound ALP antibody is then exposed to vitamin D analog coated-solid phase receptor. Solid phase is then washed and substrate reagent is added to initiate the fluorescent reaction. An inverse relationship exists between the amount of 25(OH)D in the sample and the amount of relative fluorescence units detected by the system

Precision:

Standard deviation and CV% were calculated for VIDAS® 25-OH Vitamin D Total Assay repeatability (precision within-lot, within-run, within-instrument) and reproducibility (precision between-runs, between-days, between calibrations, between-lots, between-instruments). The precision profile of the VIDAS 25-OH Vitamin D Total Assay demonstrates Total Precision CV% from

16.0% at 10.5 ng/mL to 2.4% at 119.8 ng/mL

Linearity:

The low Sample pool had an estimated concentration of 7.1 ng/mL. The High Sample pool had an estimated concentration of 132.1 ng/mL. Analysis by weighted linear regression indicated that the assay results demonstrate linearity less than 12% across the claimed range of [8.1– 126] ng/mL. Limit of Blank, Limit of Detection, and Functional Sensitivity: The LoB of the VIDAS® 25OH Vitamin D Total Assay is 6.2 ng/mL. The LoD is 8.1 ng/mL. The LoQ (functional sensitivity: CV.

25(OH)D2 cross reactivity determination:

The 25(OH)D2 cross reactivity was determined using natural non-spiked serum samples for which 25(OH)D2 and 25(OH)D3 concentrations were previously obtained by LC-MS/MS. For each sample, 25(OH)D2 cross reactivity is determined according to the equation:

$$\text{D2 cross reactivity (\%)} = \frac{[25(OH)D\,Total]_{VIDAS} - [25(OH)D3]_{LCMS}}{[25(OH)D2]_{LCMS}} \times 100$$

As

a result, 25(OH)D2 cross reactivity for VIDAS® 25-OH Vitamin D Total Assay is 91%.

Statistical Analysis

The data were recorded as mean ± **SD** with t-independent test for difference between two groups, different range test at $p \leq 0.05$ which was agreed as significant, where as highly significant when $p \leq 0.001$, and the range test at $p \geq 0.05$ was agreed as non- significant.

Result and Discussion

Table (1) shows the demographic data for (20) patients with PCOS and (20) healthy people, as well as the mean ± SD values for study parameters when compared to the control group in this study.

Table (1): Distribution of mean± SD for all variables and parameters in all Studied Groups

Parameter	G1=20 sample PCOS	G2=20 sa control gro mmple up	P-value
Age	16.62 ± 5.57	17.00	0.205

		±6.91	
BMI (Kg/m2)	18.97± 3.5	19.86 ± 3	0.294
SOD (U/ mL)	258.97±42.55	92.96± 8.70	*0.000
Vitamin D ( )	8.69±1.32	4.49±0.51	*0.000

Polycystic ovary syndrome (PCOS) is a hormonal disorder that affects women of reproductive age. It is characterized by the presence of cysts on the ovaries, irregular periods, and high levels of androgens (male hormones) in the body (22). The results of the current study showed a high level of SOD enzyme concentration in PCOS patients compared to the healthy group, while the results showed a decrease in the level of vitamin D in the patients group compared to the healthy group.

The finding of a high level of SOD enzyme concentration in PCOS patients compared to the healthy group may suggest that there is increased oxidative stress in these patients. However, more research is needed to confirm this and to understand the implications of this finding on the development and progression of PCOS (25). There have been several studies investigating the potential role of SOD in the treatment of PCOS.

Recent studies have suggested that vitamin D may also have a role in the pathophysiology of PCOS, as it has been shown to affect insulin sensitivity, ovarian function, and inflammation. The finding of a decrease in the level of vitamin D in the PCOS patient group compared to the healthy group may suggest that vitamin D deficiency may be a risk factor for the development of PCOS. However, more research is needed to confirm this and to understand the underlying mechanisms involved. The results of the previous studies showed that (22) genetic and environmental factors vitamin D deficiency caused increases in the clinical findings and in the metabolic the 35 hormonal profiles of PCOS patients, which is consistent with other studies, present study also showed that vitamin D deficiency may be a risk factor or may play a role in the pathophysiology of PCOS (23 ,24)

Overall, these findings provide insights into the potential role of oxidative stress and vitamin D in the pathophysiology of PCOS. Further research is needed to fully understand the implications of these findings and to develop new strategies for the prevention and treatment of this common endocrine disorder. Relationship between SOD and vitamin D has also been investigated in the context of PCOS, while there is some evidence to suggest that SOD may be beneficial in the treatment of PCOS, more research is needed to fully understand its effects and mechanisms of action.

Conclusion

The conclusion of this study shows that in patients with polycystic ovary syndrome (PCOS), there is a significant increase in superoxide dismutase (SOD) enzyme levels which may indicate increased oxidative stress, as well as a significant decrease in vitamin D levels compared to the healthy group. These findings indicate that vitamin D deficiency and oxidative stress may play a role in the pathophysiology of PCOS, although more research is needed to understand the underlying mechanisms and develop more effective prevention and treatment strategies for this endocrine disorder.

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