

Assessment of Malondialdehyde as a Biomarker of Oxidative Stress and Its Correlation with 25-hydroxy Vitamin D₃ in Women with Polycystic Ovary Syndrome

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Abstract

Background: Polycystic Ovary Syndrome (PCOS) is a prevalent endocrinology disorder affecting women in the years of reproduction. Malondialdehyde (MDA) is a result of polyunsaturated fatty acid peroxidation and is widely utilized as a trustworthy indicator of peroxidation of lipids and oxidative damage. Conversely, Vitamin D deficiency is a global issue affecting around one billion individuals. 25-hydroxyvitamin D₃ is linked to multiple metabolic processes and reproduction facets of women with PCOS and thus may contribute to the PCOS pathophysiology.

Objectives: To measure and compare serum malondialdehyde levels and 25-hydroxyvitamin D₃ concentrations between women with polycystic ovary syndrome (PCOS) versus age-matched healthy controls and to investigate the correlation between malondialdehyde and 25-hydroxyvitamin D₃ levels in both groups to elucidate potential mechanistic links between oxidative stress and vitamin D deficiency in PCOS pathophysiology.

Methods: The present case-control research was conducted at the Infertility Centre of Al-Batool Teaching Hospital, Diyala Governorate, Iraq, in collaboration with the Biochemistry Department at the College of Medicine, University of Baghdad, from April 2024 to January 2025. A total of 132 women aged 18-40 years were included in this study, comprising 66 PCOS cases and 66 healthy controls. A competitive enzyme-linked immunosorbent assay assessed the concentrations of serum malondialdehyde and 25-hydroxy vitamin D₃.

Results: The results indicated markedly elevated serum malondialdehyde concentrations within the PCOS group compared to the controls. The concentrations of 25-hydroxy vitamin D₃ in the PCOS group were considerably reduced compared to the controls. A significant negative correlation was found between serum malondialdehyde levels and 25-hydroxy vitamin D₃ in the PCOS Group.

Conclusion: The current study showed that PCOS cases had much higher MDA levels than controls, probably indicating that they have experienced more oxidative stress. The level of 25(OH)D showed a common lack of this vitamin in the PCOS cases.

Keywords: Body Mass Index; Malondialdehyde; Oxidative Stress; Polycystic Ovary Syndrome; Vitamin D₃ Deficiency.

Introduction:

Polycystic ovary syndrome (PCOS) is defined by hyperandrogenism, metabolic and reproductive disorders, and is linked to insulin resistance, which obesity aggravates, but is not a prerequisite for. PCOS is a prevalent endocrinal and metabolic condition caused by hereditary and environmental factors affecting women in the years of reproduction (1,2). It is defined by (A) persistent anovulation, (B) biochemical and/or clinical hyperandrogenism, and (C) polycystic ovarian morphology. PCOS has major medical consequences and can lead to health problems linked to hyperandrogenemia, increased insulin resistance (IR), cardiovascular diseases (CVDs), long-term inflammation, overweight or obesity, and malignancies. It is the primary cause of infertility

and persistent anovulation (3). Although a lot of progress has been made in understanding PCOS's symptoms, how it works, and how to treat it, problems still exist because we do not fully understand its causes and the lack of specific treatments that work for everyone (4). PCOS is a burgeoning health concern among females due to lifestyle alterations, heightened stress levels, insufficient physical exercise, and significant disruptions in menstrual cycle patterns. Currently, there is no definitive therapy for the issue, so the most efficient and important approach is to prevent its progression and facilitate early detection to avert severe long-term repercussions (5). Numerous symptoms are associated with PCOS, including acne, hirsutism, acanthosis, seborrhoea, alopecia, insomnia, infertility, and irregular menstruation (6). Worldwide, according to 1990 NIH standards, PCOS impacts 6–10% of females or

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even more people according to the broader Rotterdam criteria and Androgen Excess Society (AES) criteria, which renders it one of the more prevalent human diseases and the most pervasive endocrinopathy among reproductive-age women (7). Oxidative stress, a significant pathogenic mechanism of PCOS, transpires in several body compartments, where it begins or exacerbates cellular alterations. Various indicators were examined to assess oxidative stress in the serum, revealing elevated levels of nitric oxide (NO), xanthine oxidase (XO), and malondialdehyde (MDA) (8). Malondialdehyde refers to the extremely unstable three-carbon dialdehyde formed as a result of polyunsaturated fatty acid peroxidation and is widely utilized as a trustworthy indicator of peroxidation of lipids and oxidative damage (9,10). MDA can disrupt several physiological functions in the human body by interacting with particles, including proteins and DNA (11). Hyperandrogenism, IR, dyslipidaemia, and obesity linked to PCOS may elevate MDA levels while concurrently diminishing antioxidant enzyme concentrations. The serum concentrations of MDA indicate the degree of lipid peroxidation and damage to tissue occurring in people with PCOS (12). Deficiency with 25-hydroxyvitamin D₃ (25(OH)D₃) is a global issue affecting around one billion individuals. This deficiency or insufficiency primarily results from insufficient dietary consumption, sedentary habits, and diminished sunlight exposure (13). Vitamin D may influence reproductive operations, as vitamin D receptors (VDR) and vitamin D metabolizing enzymes have been identified in female reproductive organs. Vitamin D is linked to multiple metabolic processes and reproduction facets of women with PCOS and thus may contribute to the PCOS pathophysiology (14).

This study aimed to measure and compare serum MDA levels and 25(OH)D₃ concentrations between women with PCOS versus age-matched healthy controls and to investigate the correlation between MDA and 25(OH)D₃ levels in both groups to elucidate potential mechanistic links between oxidative stress and vitamin D deficiency in PCOS pathophysiology.

Cases and Methods:

This case-control research was conducted at the Infertility Centre of Al-Batool Teaching Hospital, Diyala Governorate, Iraq, with the Biochemistry Department, College of Medicine, University of Baghdad, from April 2024 to January 2025. One hundred thirty-two women aged 18-40 years were included in this study. The participants were categorized into two groups: Sixty-six PCOS patients and 66 healthy controls.

Inclusion Criteria: The cases were selected consecutively from the Infertility Centre after confirming the PCOS diagnosis according to the Rotterdam European Society for Human

Reproduction and before starting any PCOS medication (insulin-sensitizing drugs).

Exclusion Criteria: All patients with metabolic or endocrinal disorders, including diabetes mellitus, thyroid dysfunction, hypertension, liver disease, chronic renal disease, patients on vitamin D tablets, premature ovarian failure, virilizing adrenal, and ovarian tumors were excluded from this study. Cases with etiological factors, including prolactinoma, congenital adrenal hyperplasia, and cushing syndrome simulating PCOS, were also excluded.

We aimed for 80% power to detect a significant difference. The significance level was set at 0.05. Using these parameters, we calculated the required sample size using the G*Power software (version 3.1). The specific parameters entered into the software were (1) Effect Size d: Calculated as the mean difference divided by the standard deviation; (2) Analysis: A priori power analysis; (3) Test Family: t-tests; (4) Statistical Test: Two-sample t-test (mean difference between two independent groups); (5) Type III Error Probability (alpha): 0.05; (6) Power (1 – beta error probability): 0.80. The software output indicated that a sample size of 66 participants per group would be sufficient to achieve 80% power to detect a significant difference in MDA and vitamin D₃ levels between the groups.

Formula for Sample Size per Group (n):

$$n = \frac{2 * (Z_{1-\alpha/2} + Z_{1-\beta})^2}{d^2}$$

- $Z_{1-\alpha/2}$: Critical value for the significance level α .
- $Z_{1-\beta}$: Critical value for power $1-\beta$.
- d : Cohen's effect size.

The Ethical and Scientific Review Boards Commission of the Biochemistry Department, College of Medicine, University of Baghdad gave their approval to this research. Additionally, the Scientific Research Committee of Diyala Health Directorate, Diyala, Iraq provided their ethical clearance. Participants verbally agreed before participating in this study.

Blood samples were taken from each patient and control participant. Five milliliters (ml) of blood were aspirated from a peripheral vein during the follicular phase (second or third day of menstrual periods) and was allowed to clot for 15 minutes, then centrifuged for 10 minutes at 4000 rounds per minute (rpm). Laboratory testing included serum measurements of MDA and 25(OH)D₃, which Competitive-ELISA performed with the principle that the limited the number of antigen binding sites, forcing a target analyte and a labelled analogue to compete for antibody binding according to the manufacturer (Elabsience Company -Houston, Texas, USA). Each subject's serum was divided into two samples and then transported to a 1.5 millilitre Eppendorf tube for freezing at -80 °C until the time of the studied parameter measurements.

Statistical Analysis:

Statistical analysis was done using Microsoft Excel for organized data and Statistical Package for Social Sciences (SPSS) version 26.0. which described the data using percentages, means and standard deviation (SD). Between-group comparisons were conducted using independent samples t-tests and the Pearson Chi-square test for normally distributed variables. The equality of variances was evaluated using Levene's test, and appropriate corrections were applied when the assumption of homogeneity of variance was violated. The Pearson correlation

regression test was used to analyse the numerical data correlations. The statistical significance level was determined at $p < 0.05$. The ROC curve (receiver operating characteristic) and its area under the curve (AUC) have been used to evaluate the ability to diagnose each biomarker in differentiating between PCOS cases and their controls.

Results:

Table (1) shows that the cases and controls were not significantly different regarding age, height, weight, and body mass index and were well-matched.

Table 1: The demographic variables for patients and controls

Parameters	Group				P-value
	PCOS		Control		
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	
Age (years)	26.1 $\pm$ 4.82	18 - 39	27.1 $\pm$ 3.73	18.0 – 35.0	0.2276
Height (cm)	158.4 $\pm$ 5.57	144 - 172	159.4 $\pm$ 4.62	150.0 – 173.0	0.2637
Weight (Kg)	79.1 $\pm$ 11.43	58 – 111.1	76.8 $\pm$ 8.21	62.0 – 92.0	0.1756
BMI (Kg/m ²)	31.6 $\pm$ 4.38	25.8 - 38.9	30.3 $\pm$ 3.43	25.3 - 39.3	0.0748

BMI: Body mass index; Student's t-test was used to compare the group means

The serum MDA concentrations were markedly elevated among the PCOS group (946.3 $\pm$ 97.79 ng/ml) compared to the controls (457.4 $\pm$ 81.32 ng/ml, $p < 0.0001$). Compared to the control group, the PCOS group had significantly lower levels of serum 25(OH)D₃ (21.2 $\pm$ 1.93 ng/ml) than their

controls (37.6 $\pm$ 4.20 ng/ml) with a p-value of < 0.0001 , Table (2). The distribution of patients and controls according to serum levels of 25(OH)D₃ were shown in Table (3).

Table 2: Serum 25-hydroxyvitamin D₃ and malondialdehyde for patients and controls

Parameters	PCOS (n=66)		Control (n=66)		P value
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	
S. MDA (ng/mL)	946.3 $\pm$ 97.79	761.7-1277.9	457.4 $\pm$ 81.32	343.6-854.6	$< 0.0001^*$
S. 25-hydroxy vitamin D ₃ (ng/mL)	21.2 $\pm$ 1.93	14.2-27.7	37.6 $\pm$ 4.20	25.3-47.3	$< 0.0001^*$

*** Student's t-test was used to compare the group means**

Table 3: Distribution of patients and controls by serum levels of 25-hydroxy vitamin D₃

25-OH Vitamin D ₃ (ng/ml)	PCOS (n=66)		Controls (n=66)		P value
	No	%	No	%	
Normal (≥ 30)	4	6.0%	42	63.6%	$< 0.001^*$
Insufficiency (21 - 29)	24	36.4%	17	25.8%	
Deficiency (≤ 20)	38	57.6%	7	10.6%	

*** Pearson Chi-square test at the 0.05 level indicates a significant difference in proportions.**

Figures 1 and 2 illustrate the correlation between study parameters and BMI. They indicate an important positive correlation between BMI and serum MDA levels ($r = 0.697$, $p = 0.0001$) and a highly negative correlation between BMI and 25(OH)D₃ concentrations ($r = -0.843$, $p = 0.0001$)

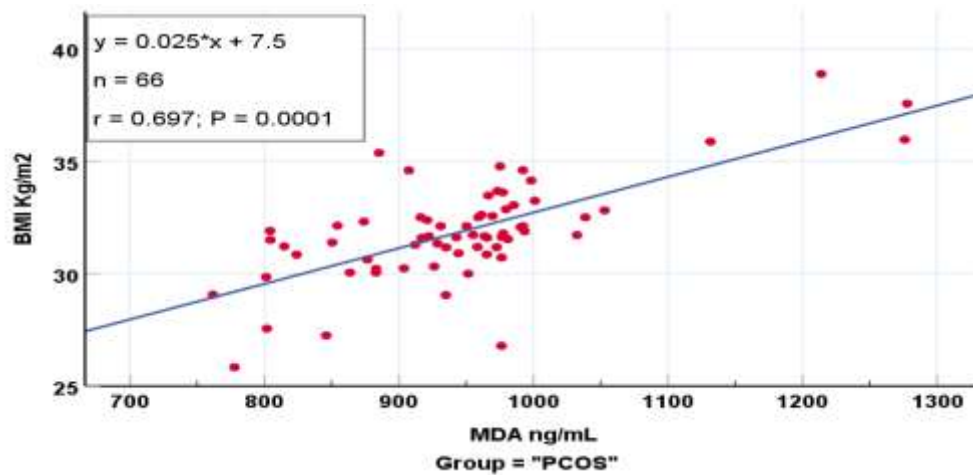


Figure 1: Scatter diagram showing the correlation between serum MDA levels and BMI in PCOS cases

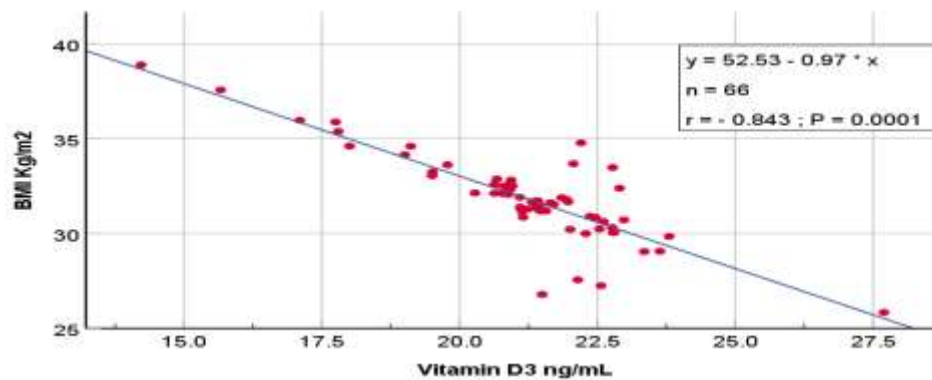


Figure 2: Scatter diagram showing the correlation between serum 25-hydroxyvitamin D₃ concentrations and BMI in PCOS cases

Figure (3) illustrates the relationship between 25(OH)D₃ concentrations and serum MDA concentrations among the PCOS cases. An inverse correlation was observed between 25(OH)D₃ concentrations and serum MDA concentrations ($r = -0.699$, $p = 0.0001$).

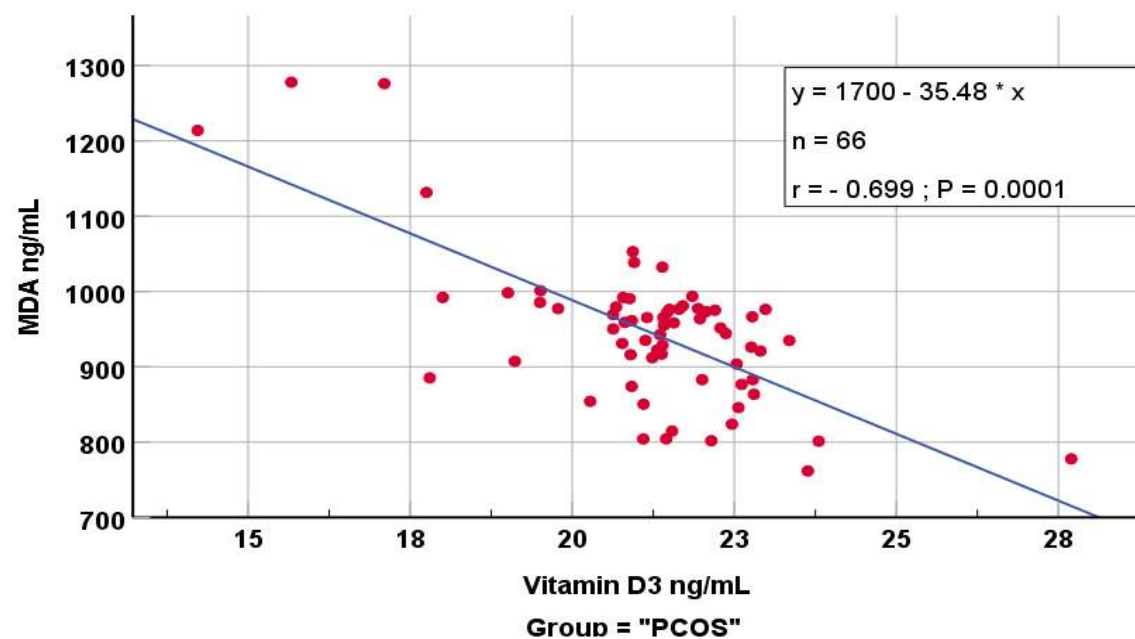


Figure 3: Scatter diagram showing the correlation between 25(OH)D₃ and MDA concentrations in PCOS cases

The results of the receiver operating characteristic (ROC) analysis and the area under the curve (AUC) assessment in differentiation between PCOS and healthy groups revealed that serum MDA shows fair diagnostic performance with an AUC of 0.960. Using a cutoff value of >528.3 (ng/mL), it achieved 87.9% sensitivity and 93.9% specificity. Serum 25(OH)D₃ demonstrates strong diagnostic performance (AUC: 0.991). Using a cutoff value of ≤26.8 (ng/mL), with 98.5% sensitivity and 98.5% specificity, indicated a very high discriminatory ability between these two groups.

Discussion:

The results of the current study demonstrated that the PCOS group had a much higher mean MDA value than the control group, probably indicating an oxidative damage and increased free radical production among PCOS subjects. Consistent with prior research, a comparison of MDA concentrations revealed significant disparities between the cases and controls (15,16). In their meta-analysis, Murri *et al.* included 1481 women (790 diagnosed with PCOS and 691 controls). They found that the mean concentration of MDA in females with PCOS were higher by about 47% compared to the controls, suggesting that concentrations of MDA may be a significant indicator for oxidative stress in PCOS (17).

The current study revealed that significantly lower levels of 25(OH)D₃ were found in women with PCOS compared to their age and BMI-matched controls. This finding is in agreement with prior researches which indicated that the notable disparity in 25(OH)D₃ concentrations among those with PCOS and their controls highlights the possible involvement of 25(OH)D₃ in the onset and aggravation of PCOS. 25(OH)D₃ deficiency was prevalent among women with PCOS, which is consistent with prior research indicating that 67% - 85% of women with PCOS have 25(OH)D₃ levels below 20 ng/ml (18). IR in PCOS, leading to high blood sugar levels and elevated free fatty acid levels, might result in heightened generation of reactive oxygen species (ROS), particularly in the presence of overweight and obesity. Due to the association of PCOS with diminished concentrations of antioxidants, it is seen as a condition that heightens the danger of oxidative stress (19). Interestingly, vitamin D is essential for numerous human physiological activities, including the prevention of inflammation along with oxidative damage (20). This study also found a high percentage of 25(OH)D₃ deficiency and 25(OH)D₃ insufficiency. These findings align with numerous research from the Middle East that indicated a significant prevalence of vitamin D insufficiency in this region (21,22). Our data aligns with a Polish investigation that revealed a significant prevalence of vitamin D deficiency. In women with PCOS about a third exhibited insufficient vitamin D levels, a sixth had normal levels, very few had elevated levels, and none reached potentially toxic levels of vitamin D

(23). There are several potential causes of vitamin D deficiency, including dark skin tone, insufficient sun exposure, vegetarian diets, and a lack of vitamin D-rich foods (24). Our data indicate that addressing deficiencies in vitamin D could serve as a helpful strategy in the management of PCOS.

The study results revealed strong positive correlations between blood MDA levels and BMI among the PCOS group, corroborating earlier research indicating that MDA levels with the obese PCOS cohort were higher than in the non-obesity group. We believe that this heightened oxidative stress resulted from obesity (25,26).

In the presented work, a negative correlation occurred between serum 25(OH)D₃ concentrations and BMI in the PCOS group which is consistent with Nowak *et al.* (23) and Siahaan *et al.* (27). On the other hand, Alawad (28) and Shilpasree *et al.* (29) suggested that diminished vitamin D concentrations in PCOS cases is attributable to obesity, which is predominantly linked to this disease, as vitamin D in obese women is sequestered in adipose tissue, resulting in reduced vitamin D levels in body fluids.

The current study revealed a significant inverse correlation between the concentrations of MDA and concentrations 25(OH)D₃. No information exists regarding the correlation between 25(OH)D₃ concentrations and oxidative damage markers. A prior investigation examined the relationships between serum 25(OH)D₃ and MDA levels as well as protein carbonyl (PC) in women with PCOS, but no relationship was found between levels of vitamin D in the blood and the aforementioned oxidative stress indicators (30).

Limitations:

The limited diversity in the study population regarding ethnicity and socioeconomic status may reduce the generalizability of the results.

Conclusion:

The current study showed that PCOS cases had much higher MDA levels than controls, probably indicating that they have experienced more oxidative stress. Vitamin 25(OH)D₃ levels showed a common lack of this vitamin in the PCOS cases.

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Authors' declaration:

We confirm that all the figures and tables in the manuscript belong to the current study. Authors sign on ethical consideration's approval-ethical clearance: The project was approved by the local ethical committee in the place where the research was

conducted, or samples collected and treated according to the code number (13) on (6/4/ 2025).

Conflict of interest: None

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Data availability:

Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Authors' contributions:

Study conception & design: (Halla Ghazi Mahmood, Mohammed Naji Qasim). Literature search: (Mohammed Naji Qasim, Halla Ghazi Mahmood). Data acquisition: (Mohammed Naji Qasim, Halla Ghazi Mahmood). Data analysis & interpretation: (Halla Ghazi Mahmood, Mohammed Naji Qasim). Manuscript preparation: (Mohammed Naji Qasim, Halla Ghazi Mahmood). Manuscript editing & review: (Halla Ghazi Mahmood, Mohammed Naji Qasim).

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دور المألونديالدهيد كمؤشر للإجهاد التأكسدي لدى النساء المصابات بمتلازمة تكيس المبايض وارتباطه بـ 25-هيدروكسي فيتامين د3

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¹إفـرع الكيمياء الحياتية، كلية الطب،/جامعة بغداد، بغداد، العراق.

الخلاصة:

الخلفية: تمثل متلازمة تكيس المبايض اضطراباً غدياً صماءً شائعاً يصيب النساء في مرحلة الإنجاب. ينتج المألونديالدهيد نتيجة أكسدة الأحماض الدهنية المتعددة غير المشبعة، ويستخدم على نطاق واسع كمؤشر موثوق لأكسدة الدهون والضرر التأكسدي. من ناحية أخرى، يعد نقص فيتامين د مشكلة عالمية تسبب حوالي مليار شخص. يرتبط فيتامين د بالعديد من العمليات الأيضية والجوانب التناسلية لدى النساء المصابات بمتلازمة تكيس المبايض، وبالتالي قد يساهم في الفيزيولوجيا المرضية لمتلازمة تكيس المبايض.

الأهداف: قياس ومقارنة مستويات المألونديالدهيد في مصل الدم وتركيزات 25-هيدروكسي فيتامين د3 لدى النساء المصابات بمتلازمة تكيس المبايض مع مجموعة من النساء الأصحاء المتطابقات في العمر. وبحث في العلاقة بين مستويات المألونديالدهيد و 25-هيدروكسي فيتامين د3 في كلا المجموعتين لتوضيح الروابط الميكانيكية المحتملة بين الإجهاد التأكسدي ونقص فيتامين د في الفسيولوجيا المرضية لمتلازمة تكيس المبايض.

المنهجية: أجريت دراسة الحالات والشواهد الحالية في مركز العقم بمستشفى البتول التعليمي في محافظة ديالى بالعراق، مع قسم الكيمياء الحيوية بكلية الطب جامعة بغداد، من نيسان 2024 إلى كانون الثاني 2025. شملت هذه الدراسة ما مجموعه 132 امرأة تتراوح أعمارهن بين 18 و 40 عاماً، تضمنت 66 حالة مصابة بمتلازمة تكيس المبايض، و 66 امرأة سليمة كمجموعة ضابطة. وقد قيم تركيز المألونديالدهيد و 25-هيدروكسي فيتامين د3 في المصل باستخدام اختبار الممتز المناعي المرتبط بالإنزيم التنافسي.

النتائج: أشارت نتائج البحث إلى ارتفاع ملحوظ في تركيزات المألونديالدهيد في مصل الدم لدى مجموعة متلازمة تكيس المبايض مقارنة بالمجموعة الضابطة. انخفضت تركيزات و 25-هيدروكسي فيتامين د3 داخل مجموعة متلازمة تكيس المبايض بشكل كبير مقارنة بتلك الموجودة في المجموعة غير المصابة بمتلازمة تكيس المبايض، مما كشف عن وجود ارتباط سلبي كبير بين مستويات المألونديالدهيد في المصل و 25-هيدروكسي فيتامين د3 ($P < 0.0001$, $r = -0.699$) في مجموعة متلازمة تكيس المبايض.

الاستنتاج: تظهر الدراسة الحالية أن حالات متلازمة تكيس المبايض كان لديها مستويات مألونديالدهيد أعلى بكثير من العينة الضابطة، مما يشير على الأرجح إلى تعرضهن لمزيد من الإجهاد التأكسدي. تظهر مستويات فيتامين د نقصاً شائعاً في هذا الفيتامين في حالات متلازمة تكيس المبايض.

مفتاح الكلمات: مؤشر كتلة الجسم؛ مألونديالدهيد؛ الإجهاد التأكسدي؛ متلازمة تكيس المبايض؛ نقص فيتامين د3.