مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الخامس والعشرون - المجلد الخامس - علوم الحياة - آذار 2025 م
doi.org/10.52866/esj.2025.05.25.21

Association of melatonin in women with polycystic ovary syndrome

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Abstract:

Melatonin is a tryptophan derivative, involved in different biological functions, including glycemic status, lipid profile and oxidative stress status. These features are important for the pathogenesis of polycystic ovary syndrome (PCOS). The present study aimed to evaluate the serum melatonin level of women with PCOS and its association with its management. Case-control study was managed in the Gynecology Consulting Clinics in Kirkuk and Erbil Governorate Hospitals, Northern Iraq, from December 2023 to June 2024. The study involved 90 participants aged 18-45 years, including 45 women diagnosed with severe polycystic ovary syndrome based on the International Evidence-based Guideline (2023) and an equal number of matched healthy women controls. Serum levels of melatonin, oxidative stress parameters, testosterone and metabolic variables, were assessed using the ELISA (enzyme-linked immunosorbent assay), Electrochemiluminescence based immunoassay (ELICA) using Cobas e411 and colorimetric kits. In this study, ROC curve analyses, correlation coefficients, and descriptive statistics were employed. Serum melatonin level was statistically different between PCOS and control (163.31±57.74; 290.74±107.74) respectively. Biochemical, hormonal and metabolic variables had significant correlations with melatonin in women with PCOS. The AUC of melatonin was 0.864 (p =0.001), demonstrating that, in comparison to healthy control women, it has the proper sensitivity to detect PCOS patients. These finding suggest that melatonin had a significant association with PCOS and could be used as a useful biomarker of PCOS.

Keywords: melatonin, PCOS, oxidative stress, testosterone, metabolic variables.

ارتباط الميلاتونين لدى النساء المصابات بمتلازمة تكيس المبايض

مهاباد حسين رسول[1] ، فائزة ناصر طعمة[1] ، صالح محمد رحيم[1,2]
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مستخلص:

الميلاتونين هو أحد مشتقات التربتوفان، ويشارك في وظائف بيولوجية مختلفة، بما في ذلك وضع السكر ومستوى الدهون والإجهاد التأكسدي. هذه السمات مهمة لتطور متلازمة تكيس المبايض (PCOS). تهدف الدراسة الحالية إلى تقييم مستوى الميلاتونين في مصل النساء المصابات بمتلازمة تكيس المبايض وارتباطه بإدارتها. تم إجراء دراسة الحالة الضابطة في عيادات استشارية أمراض النساء في مستشفيات محافظة كركوك وأربيل، شمال العراق، من ديسمبر 2023 إلى يونيو 2024. وشملت الدراسة 90 مشاركة تتراوح أعمارهن بين 18 و 45 عامًا، تضمنت 45 امرأة تم تشخيصهن بمتلازمة تكيس المبايض الشديد بناءً على المبادئ التوجيهية الدولية القائمة على الأدلة (2023) يقابلهن عدد متساوٍ من النساء السليمات كمجموعة ضابطة. تم تقييم مستويات الميلاتونين ومعايير الإجهاد التأكسدي والتستوستيرون والمتغيرات الأيضية في المصل باستخدام اختبار ELISA (اختبار الامتزاز المناعي المرتبط بالإنزيم)، واختبار التألق الكيميائي الكهربائي المناعي(ELICA) باستخدام Cobas e411 وعدد القياس اللوني. في هذه الدراسة، تم استخدام تحاليل منحنى ROC ومعاملات الارتباط والإحصاءات الوصفية. كان مستوى الميلاتونين في المصل مختلفًا إحصائيًا بين متلازمة تكيس المبايض والمجموعة الضابطة (163.31 ± 57.74؛ 290.74 ± 107.74) على التوالي. كان للمتغيرات الكيميائية الحيوية والهرمونية والأيضية ارتباطات مهمة بالميلاتونين لدى النساء المصابات بمتلازمة تكيس المبايض. قدرت المساحة تحت المنحنى للميلاتونين ب 0.864 (p=0.001)، مما يدل على أنه بالمقارنة مع النساء السليمات، يتمتع الميلاتونين بالحساسية المناسبة للكشف عن مرضى متلازمة تكيس المبايض. تشير هذه النتائج إلى أن الميلاتونين كان له ارتباط كبير بمتلازمة تكيس المبايض ويمكن استخدامه كعلامة حيوية مفيدة لمتلازمة تكيس المبايض.

الكلمات المفتاحية: الميلاتونين ، متلازمة تكيس المبايض ، الاجهاد التأكسدي ، هرمون الشحمون الخصوي ، متغيرات الايض.

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder, affecting roughly 6-15% of females in their reproductive age between 15-45 years globally [1,2]. Based on data from the World Health Organization (WHO), the prevalence of PCOS varies depending on the community under study [3]. It is distinguished by a diverse array of clinical manifestations, including inconsistent menstrual periods, elevated androgen levels, hirsutism and the ultrasonographic detection of polycystic ovaries [4,5]. In addition, two of the last three characteristics are included in the Rotterdam criteria, which are the most widely used diagnostic standards: (a) polycystic ovaries, (b) oligo- or an-ovulation, and (c) hyperandrogenism [6,7]. The majority of patients have metabolic disorders, the most important of which are obesity, insulin resistance, increased risk of type 2 diabetes, cardiovascular diseases, chronic inflammation, and high oxidative stress [8,9,4]. Several investigations have demonstrated that oxidative stress plays a significant role in the pathophysiology of PCOS and that oxidative circulation indicators are higher than normal in PCOS patients [10]. These pathophysiologies associated with PCOS are interrelated with each other. The molecular etiology of PCOS is still unknown, despite mounting evidence that genetic and environmental variables play a role in the pathogenesis of PCOS [11].

An imbalance between the body's enzymatic and non-enzymatic antioxidant output and oxidant generation is known as oxidative stress, resulting in excessive levels of reactive oxygen species (ROS). Chronic hyperinsulinemia, which is believed to be caused by insulin resistance, impairs follicular growth, changes the gonadal response, and impairs ovarian androgen metabolism [12]. Insulin resistance has been linked to oxidative stress and ROS generation. Reactive oxygen species generation is elevated in response to PCOS, and a number of characteristics of PCOS, including as obesity, insulin resistance, and androgen excess, may exacerbate oxidative stress in these individuals [13].

Melatonin is a tryptophan derivative, is the sole hormone that vertebrates' pineal glands manufacture

and secrete, and it plays a role in controlling circadian rhythm. Melatonin is involved in several biological functions, namely glycemic status, lipid profile, inflammatory parameters and anti-oxidative status [14,15]. Moreover, melatonin is a strong antioxidant and free radical scavenger that targets the mitochondria, preventing oxidative stress and lowering reactive oxygen species (ROS) and reactive nitrogen species (RNS) locally. Melatonin can also neutralize free radicals and exert reproductive effects, thus improving oocyte quality [16,17]. The purpose of this study was to assess melatonin plasma levels, its correlation, and the potential for using it as a PCOS biomarker.

Participants and Methods

Study population

Gynecology Consulting Clinics in Erbil Governorate Hospitals, Northern Iraq, served as the site of this case-control study, for the period from December 2023 to June 2024. The study was based on dealing with 90 married women aged 18-45 years, 45 women patients in a case of severe affected with polycystic ovary syndrome were diagnosed based on the International Evidence-based Guideline [18]. The diagnosis of PCOS was confirmed using information from the history, physical examination, biochemical tests, and ultrasound results. On the other hand, 45 women joined the healthy group as a control group consisting of healthy women with regular menstrual cycles and normal ovaries as determined by ultrasound as well as normal biochemical and clinical examinations and they were considered free from PCOS.

Anthropometric evaluation

Baseline characteristics were recorded including age, height and weight. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

Blood samples collection and biochemical variable examination

After advising the participants to fast overnight before to the blood draw, venous blood collection was carried out early in the morning, between 8 and 9 AM, then allowing the blood in gel tubes to coagulate for 20 minutes at room temperature. To avoid losing any analytes, blood samples were placed on ice right away and separated in a chilled centrifuge. Plasma samples were then stored at -20°C. Laboratory tests were conducted for both groups

to determine the level of melatonin, glutathione, catalase, malondialdehyde and 8-OHdG by using ELISA kits Elk. Biotechnology- China). Insulin level was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Elk. Biotechnology- China). Fasting blood sugar (FBS) was measured using colorimetric kits (BIO LABO, France). Testosterone and luteinizing plasma levels was estimated by Electrochemiluminescence based immunoassay (ELICA) using Cobas e411 instrument (Roche, Germany). Insulin resistance was measured using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) calculation, which was FPG (mg/dl) × fasting insulin (**µU**/ml)/405.

Statistical analysis

The data have been statistically analyzed using SPSS software version 27(SPSS, Inc.), Analysis of Variance (ANOVA) test was used in order to evaluate the significance of variability among PCOS women and control groups and association among variables in the study. The multiple comparisons between the means are analyzed by Duncan multiple range and post HOC test and a value of P<0.05 is statistically consider significant. The correlations between melatonin and other biochemical parameters were measured by Pearson's correlation tests. Data expressed as mean ± SD [19]. To evaluate the discriminating between individuals with and without PCOS, a receiver operating characteristic (ROC) curve analysis was conducted, and the area under the curve was computed. If $p < 0.05$, the difference was deemed statistically significant.

Results

The present study enrolled 90 women, with 45 diagnosed with PCOS and 45 healthy, non-infertile women functioning as a control group. Table (1) presents the parameters characteristics of the sample of women from both the healthy control group and the group diagnosed with PCOS. There was a significant difference (P>0.05) in the ages of the PCOS patients (32.33± 7.24 years) and the control group (27.96± 5.61 years).

Table (1) Parameters characteristics of the study population

Parameters	Control	PCOS	p.value
Age years	27.96±5.61	32.33±7.24	≤0.05
BMI kg/m^2	26.19±3.23	34.62±5.49	≤0.001
Melatonin U/mL	290.74±107.74	163.31±57.74	≤0.001
8-OHdG nmol/ml	0.34±0.11	0.75±0.27	≤0.001
MDA ng/mL	2.65±.35	5.85±.49	≤0.001
Testost. ng/mL	0.35±0.19	0.58±0.64	≤0.05
Insulin µU/ml	10.44±3.41	16.91±5.94	≤0.001
FBG mg/dl	91.36 ±6.10	123.11±16.69	≤0.001
GSH ng/ml	0.20 ±0.13	0.12±0.14	≤0.05
CAT pg/mL	551.58 ± 83.37pg/mL	326.66± 60.58 pg/mL	≤0.001
HOM-IR	2.46±0.81	9.7±4.25	≤0.001

However, compared to the control group (26.19±3.23 kg/m2), the PCOS patients' BMI (34.62±5.49 kg/m2) was considerably higher (p≤0.001). PCOS women's blood melatonin levels were substantially (P≤0.001) lower (163.31±57.74 U/mL) than those of the healthy control group (290.74±107.74 U/mL). Furthermore, 8-OHdG and MDA level were significantly (p≤0.001) elevated in the serum of PCOS women (0.75±0.27 nmol/ml; 5.85±.49 ng/mL) in comparison with control (0.34±0.11 nmol/ml; 2.65±.35 ng/mL) respectively. On the other hand, GSH and CAT (0.12±0.14 ng/ml; 326.66 pg/mL) were decreased significantly (p≤0.001) compared with control (0.20 ±0.13 ng/ml;551.58 pg/mL) respectively. Testosterone and LH levels were increased significantly (p≤0.05) in PCOS women (0.58±0.64 ng/mL; 10.64± 6.51 mlU/ml) compared with control group (0.35±0.19 ng/mL; 8.40± 3.41 mlU/ml) respectively. The results revealed considerable disparities in the level of insulin, HOMA-IR and FBG parameters (p≤0.001) in PCOS group (16.91±5.94 µU/ml; 9.7±4.25;123.11±16.69 mg/dl) compared to control (10.44±3.41 µU/ml; 9.7±4.25; 91.36±6.10 mg/dl) respectively. Figure (1) shows the correlation data between melatonin and parameters involved. Except HO-

MA-IR, the findings showed a significant inverse relationship between BMI and plasma melatonin level (-0.461, p=0.001), MDA (-0.383, *p*=0.009), 8-OHdG (-0.463, *p*=0.001), Testosterone (-0.451, *p*=0.002), LH (-0.298, *p*=0.047), and metabolic parameters, Insulin (-0.362, p=0.015), FBG (-0.450, *p*=002). In return, a significant positive correlation with GSH (0.383, p=0.009) and CAT (0.493, *p*=0.001).

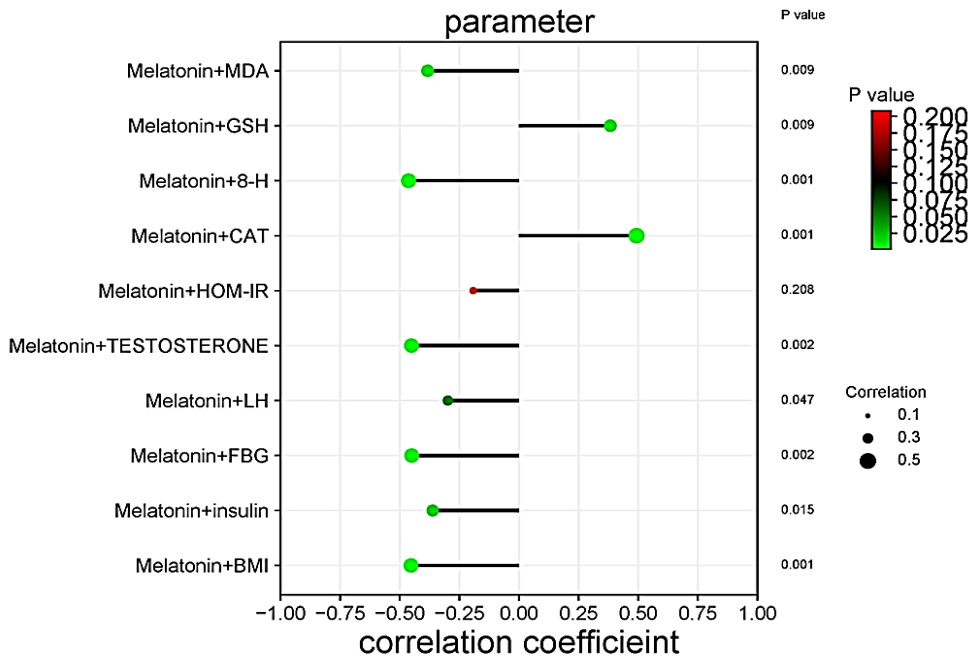

Figure (1) Correlations of plasma melatonin levels with study parameters

To investigate the effectiveness of melatonin level as biomarker for predicting PCOS, in this performed ROC analyses in PCOS patients, melatonin exhibited an appropriate diagnostic efficacy. Melatonin's cutoff value of 198.62 ng/L had a suitable sensitivity (0.756) and specificity (0.756), according to ROC curve analysis. The area under curve 0.864 (95% CI) was 0.895 (0.518 to 0.764), p = 0.001 (Fig 2).

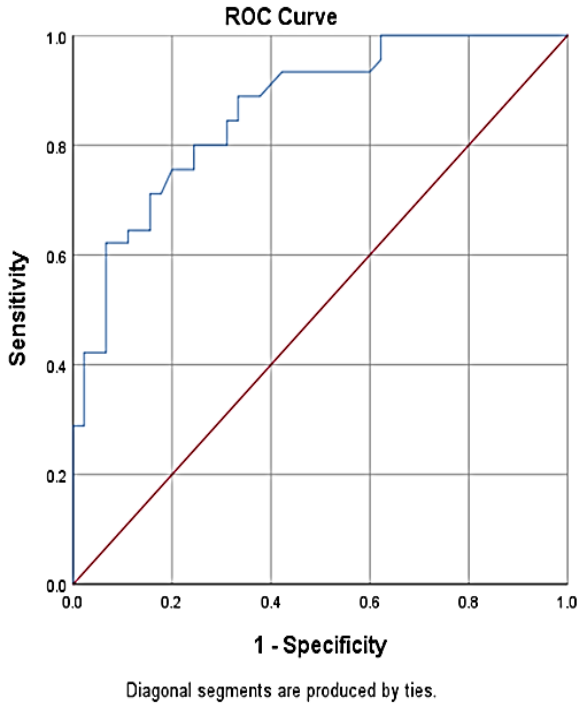

Figure (2): ROC curve of melatonin for diagnosing women PCOS

Discussion

There are two main sources of melatonin production, the first in the pineal gland with a rhythm production into the blood and cerebrospinal fluid, and the second is constituting the bulk produced from several tissues, its action is in determining redox homeostasis [14]. In the present study, we measured the concentration of plasma melatonin of PCOS and controls. In addition, correlations between melatonin and serum hormones, metabolic and oxidative stress parameters were analyzed. Our results showed a significant decrease ($p \leq 0.001$) in the level of melatonin in PCOS women as compared with control that were (163.31±**57.7**4, 290.74± 107.74) U/ml respectively. These results consisted with [20,21]. Melatonin is normally synthesized in the ovary, as both melatonin synthesizing enzymes AANAT (arylalkyl amine Nacetyltransferase) and HIOMT (hydroxy indoleOmethyl transferase) are present in ovarian tissue [22]. The decreased plasma melatonin levels are due to the decreased level of its synthesis in the ovary or its depletion caused by vital role as an

antioxidant to reduce the formation of free radicals and their end-products such as lipid peroxidation and other associated complications in women with PCOS. Li et al., [20] showed a lower concentration of melatonin in the ovarian microenvironment of women with PCOS than that of healthy women. In addition, Brzezinski et al., [23] showed that the concentration of melatonin in follicular fluid is positively correlated with serum. Therefore, the decreased serum concentration is related to the decreased melatonin in follicular fluid through its role as a free radical scavenger to reduce oxidative stress in local tissues, in order to support follicular growth by stabilizing the local ovarian microenvironment [24,25]. Oxidative stress has been previously investigated in women with PCOS, with studies showing increased lipid peroxidation, xanthine oxidase and peroxynitrites, while decreased enzymatic and non-enzymatic antioxidants were observed [26,27] which is in line with the current study's findings (Table 1). By raising mRNA levels and GSH reductase as well as the activity of many antioxidant enzymes, including glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD), melatonin indirectly activates the body's antioxidant defense mechanism, which catalyze the conversion of O2 to H2O2 [28,29]. Assessing these enzymes aids in confirming melatonin's antioxidant benefits in cells. Additionally, melatonin consumption has been linked to lower MDA levels, an indirect sign of elevated ROS generation in cells and a persistent byproduct of lipid oxidation. Therefore, the administration of melatonin influences the reduction of cell death and lipid and peroxide levels [30]. In this study, the results of the correlations between melatonin and oxidative stress variables proved a significant negative correlation with antioxidants such as GSH and CAT and a significant positive correlation with MDA and 8-OHdG, which indicates the role of oxidative stress as a cause of the PCOS and the role of melatonin in suppressing the generation of ROS resulting from the increased level of metabolic variables and androgens in PCOS women, which is what the results of the study indicated. The present study showed an increase in BMI in women with PCOS, which was indicated in previous studies [31,32], and

that it is negatively and significantly associated with plasma melatonin concentration (Fig.2). Women with PCOS, which have a high BMI, showed also an increasing in the level of testosterone and luteinizing hormone which correlates negatively with plasma melatonin level. Additionally, increasing body mass index (BMI) and abdominal fat distribution linearly increase the risk of type 2 diabetes due to changes in adipose tissue biology that link obesity to insulin resistance and beta cell dysfunction. Our study results showed a significant increasing in insulin, insulin resistance and FBG in PCOS women compared to healthy control consisting with several studies [33,34]. Besides the BMI, PCOS women were had a negatively correlation between melatonin and insulin and FBG. Thus, melatonin deficiency appears to have broad implications for the pathophysiology of PCOS, and women with PCOS are often sub-fertile secondary to ovulatory dysfunction, impaired quality of oocyte, and low endometrial receptivity [35].

Studies have suggested that melatonin may play an important role in the pathogenesis of PCOS and premature ovarian degeneration through its antioxidant role and may be associated with follicle development and oocyte quality, interfering with processes such as oocyte maturation and ovulation [16]. Melatonin has been found to exert neuroendocrine control experimentally by directly affecting GnRH cells in the hypothalamus either by reducing gene expression or by regulating G protein-coupled melatonin receptors [36]. High levels of melatonin suppress the GnRH pulse in the hypothalamus, causing a series of changes in reproductive function. There is also a direct effect on the pituitary gland to suppress the secretion of LH and FSH [37]. Thus, this finding suggests that low melatonin levels are associated with increased levels of both LH and FSH as shown in the study results (Table 1; Fig. 1). In contrast, the study by [32,37] showed that melatonin treatment improves the status of women with PCOS, decreases the pro-inflammatory condition, lowers obesity, boosts the quantity of mature eggs, and improves the quality of eggs and embryos.

Numerous studies have shown that melatonin lowers obesity, improves the proinflammatory state that drives the

development of metabolic variables like insulin and FBG, and restores adipokine patterns [21]. Together, these results suggest that melatonin may be useful in the treatment of PCOS patients because of its aromatase-modulating properties, ability to reduce hepatic gluconeogenesis, amelioration of the pro-inflammatory state associated with PCOS, enhancement of peripheral tissue glucose uptake, and consequent decrease in insulin levels. In addition, As illustrated in fig (2), our findings regarding ROC curve analysis indicated that melatonin can be utilized as a suitable sensitive biomarker in the diagnosis of PCOS.

In conclusion, Obesity was seen in women with PCOS who participated in this investigation, elevated levels of testosterone and luteinizing hormone, metabolic abnormalities, and oxidative stress, indicating disturbances in several organ systems. The present study also demonstrated that melatonin has excellent sensitivity and appropriateness in diagnosing PCOS. Therefore, based on its data, the study suggests the use of melatonin to normalize the studied basic parameters of the syndrome in women.

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