Association of Sestrin-2 for 8-hydroxy-20-deoxyguanosine and testosterone hormone level in patients with Polycystic Ovary Syndrome

Mahabad H Rasoul[1,a)], Faiza N Tomma[1,b)], Saleh M Rahim[1,c)]

1 Department of Biology, College of Science, Kirkuk University

2 Al-Qalam University College, Kirkuk, IRAQ

Abstract: Approximately 6% to 20% of premenopausal women are affected with polycystic ovarian syndrome (PCOS), making it one of the most common endocrine disorders in reproductive-age women. Among the three types of stress-responsive proteins that have persisted through evolution, Sestrin2 (SESN2) is present in the vast majority of vertebrates**.** Sestrin2, a recently discovered protein that is induced by a variety of stress conditions such as oxidative stress, inflammation, and DNA damage, is involved in the control of many vital cellular functions. The study aims to evaluate the interrelation between Sestrin2 and 8-hydroxy-20-deoxyguanosine (8-OHdG) as well as testosterone hormone levels in polycystic ovarian syndrome women (PCOS). 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 and 45 women were incorporated into the control group. Sestrin2, 8-OHdG, and testosterone levels in the PCOS cohort (298.09 pg/mL, 0.75 nmol/ml, 0.58 µU/ml) pg/mL were bring down significantly compared to control group (519.61, 0.34, 0.34) pg/mL, respectively. There was a significant difference in the level of metabolic variables and GSH between PCOS and control groups. A negetive significant correlation was showen between Sestrin2 and 8-OHdG, testosterone, BMI and metabolic variables in PCOS women, while a significantt and positve correlation was observed with GSH. A cutoff value of 373.4 ng/L showed sufficient sensitivity (0.778%) and specificity (0.756%) for differentiating between women with and without polycystic ovary syndrome (PCOS), according to the ROC curve analysis. The area under the curve was 0.895 (95% CI: 0.518 to 0.764), and the p-value was 0.036. When comparing the two groups, a striking difference was seen in terms of anthropometric traits and plasma levels of biochemical markers. Association of Sestrin2 with oxidative stress indicators like 8-OHdG and with testosterone levels in women with PCOS highlights its potential as a biomarker for oxidative stress. Additional studies required for specifying processes connecting Sestrin2 to oxidative damage and to investigate the potential therapeutic implications of targeting Sestrin2 in PCOS.

Key words: Sestrin2, 8-OHdG, Testosterone, Polycystic ovary syndrome, Oxidative stress

INTRODUCTION

Polycystic ovarian syndrome (PCOS) represents a one of the more prevalent endocrine illnesses among reproductive-aged women, impacting 5 to 20% of premenopausal females [1]. It is chiefly defined by reproductive anomalies, increased level of testosterone, and disrupted ovulation [2, 3]. The specific cause of PCOS is not fully understood, although studies suggest that hyperandrogenism plays a crucial role in this disorder. Sestrin2 (SESN2) belongs to an evolutionary preserved family of stress-responsive proteins present in most vertebrates, with three variants: SESN1, SESN2, and SESN3. SESN2 is involved in multiple pathogenic processes, such as genetically toxic stress, a lack of oxygen ,oxidative stresses, stress of the endoplasmic reticulum, inflammatory processes, and cellular death [4]. Sestrin2 has exhibited modified expression in various illnesses in humans, encompassing obese, cardiac, pulmonary, liver, kidney, neurological, and immunological diseases [5]. The Sestrin2 protein was considered essential for stress adaption by activating and augmenting the response to antioxidants via several pathways,

including p53 [6-9] Sestrin2 protein has been shown to be connected to oxidative stress and may signify heightened oxidative stress in PCOS, and is also useful in evaluating metabolic abnormalities related to PCOS.

The primary biomarker for oxidative DNA damage and a key indicator of oxidative stress is 8-Hydroxy-20-deoxyguanosine (8-OHdG) [10]. Reactive oxygen species (ROS) can interact with DNA nucleobases to form 8-OHdG, which can lead to reduced base-pairing fidelity, incorrect interpretation of neighboring pyrimidines, and the insertion of adenine across from the lesions, among other potential effects. Since 8-OHdG may be removed from DNA through base excision repair (BER), its concentrations in serum or urine can be easily measured [11]. Cardiovascular illness, cancer, and metabolic diseases are among the modern disease pathologies associated with increased 8-OHdG concentrations [12]. Oxidative stress may be linked to the development resistance to insulin and several metabolic disorders in patients with PCOS [13]. Numerous studies indicate that women suffering from PCOS exhibit elevated levels of 8-OHdG compared to healthy controls, hence reinforcing the hypothesis that oxidative stress contributes to the pathophysiology of PCOS [10, 14]. The investigation of the correlation among levels of Sestrin2 and 8-OHdG in women with polycystic ovarian syndrome (PCOS) was a prominent area of research due to its significance in mitigating oxidative stress. PCOS can be defined by an excess of androgens, resulting in symptoms including acne, hirsutism, and irregular menstrual cycles [15]. Women suffering from PCOS have an increased risk of developing metabolic syndrome and its repercussions due to high levels of androgen and related metabolic disorders, including resistant to insulin [16]. The intricate nature of PCOS is evidenced by the correlation between elevated androgen levels and heightened oxidative stress; hence, more exploration of how these interconnected elements influence PCOS is necessary [17]. This study attempted to elucidate the relationship among androgen hormone levels, serum Sestrin-2 concentrations, and 8-hydroxy-20-deoxyguanosine in patients with PCOS .

MATERIALS AND METHODS

Study population

The case-control research was performed in the Gynecologic Consulting Clinics at Kirkuk and Erbil Governorate Hospitals, North Iraq, from the month of December 2023 until June 2024. The study involved 90 participants aged 18-45 years, including 45 women diagnosed with severe PCOS based on the AE-PCOS Society criteria, which stipulate that PCOS is distinguished by a high level of androgen, ovarian disorder, and absence of associated illnesses [18]. The diagnosis of PCOS was confirmed via data collected from the history, physical examination, biochemical testing, and ultrasound reports. Conversely, 45 women were incorporated into the control group, including healthy individuals with normal menstrual cycles and normal ovaries, as confirmed by ultrasound, alongside normal biochemical and clinical evaluations, thereby categorizing them as free from PCOS.

Anthropometric evaluation

Age was one of the baseline factors recorded. We measured the participants' height and weight, and then we divided the weight by the square of the height, to get their body mass index (BMI) (kg/m^2).

Collection of blood samples and analysis of biochemical variables

Venous blood collection occurred in the morning between 8 and 9 AM, following an overnight fasting period for participants prior to the draw, and the blood in gel tubes was permitted to clot for 20 minutes at room temperature. Plasma samples were stored at −20°C after being centrifuged on ice to prevent analyte loss. Both groups were tested for sestrin2, GSH and 8-hydroxy deoxyguanosine using BT (Bioassay Technology Laboratory) ELISA kits from China. Insulin levels were measured using a Chinese Elk Biotechnology ELISA kit. Colorimetric kits from BIO LABO, France, measured fasting blood sugar (FBS). The Cobas e411 system (Roche, Germany) measured plasma testosterone. The formula for the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) indicates insulin resistance, which is FPG (mg/dl) x fasting insulin (µU/ml)/405.

Statistical analysis

Utilizing the Analysis of Variance (ANOVA) test, the statistical examination of the data was conducted with SPSS software version 27 (SPSS, Inc.). The purpose was to determine the importance of variability between the PCOS and control groups. To determine how Sestrin2 related to the research variables, the Pearson correlation was used. By measuring the area under the curve (AUC), the receiver operating characteristic (ROC) curve was used to evaluate Sestrin2 for diagnostic purposes in PCOS patients.

RESULTS AND DISCUSSION

The current study comprised 90 women, with 45 diagnosed with PCOS and 45 healthy, non-infertile women functioning as a control group. Table (1) presents the anthropometric and biochemical findings of the sample of women from both the healthy control group and the group diagnosed with polycystic ovarian syndrome. The anthropometric data indicate a considerable difference between the two research groups based on age. The body mass index also indicates a substantial disparity between the healthy cohort and the cohort of patients with PCOS. A substantial difference in sestrin2 and 8-OHdG was identified in the group of women with PCOS compared to the healthy group. Furthermore, the results demonstrated a considerable disparity in FBG, insulin hormone, HOMA-IR and GSH between the two studied demographic groups. The investigation revealed considerable disparities in testosterone levels between the two groups.

TABLE 1. Anthropometric and biochemical results of control and PCOS patient's groups

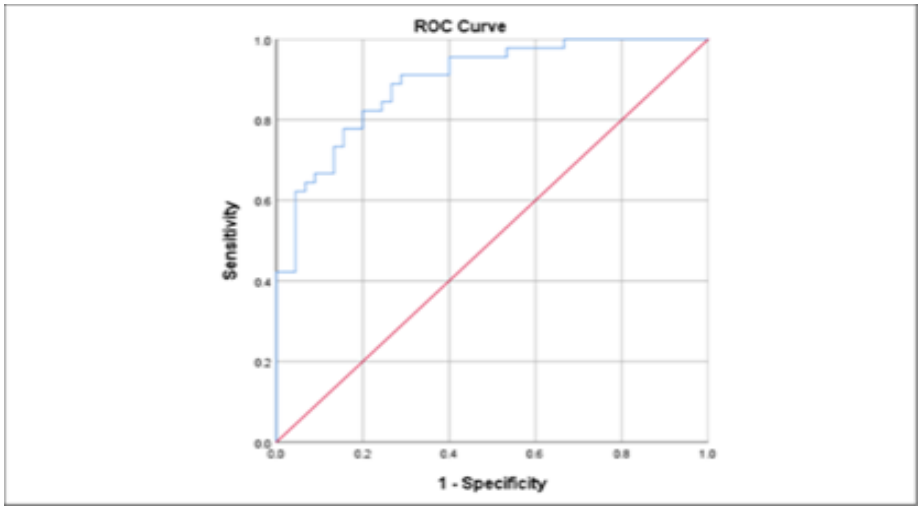
Parameters	Population groups				
	Control (n=45)		Patient(n=45)		
	Mean	Std. Deviation	Mean	Std. Deviation	P.value
Agey years	27.96	5.608	32.33	7.236	≤0.05
BMI kg/m2	26.1180	3.22669	34.6213	5.48826	≤0.001
Sestrin 2 pg/mL	509.8180	151.80091	333.2271	205.18014	≤0.001
8-OHdG nmol/ml	.3447	.11210	.7489	.27222	≤0.001
HOM-IR	2.4600	.80577	9.7682	4.25107	≤0.001
Testosterone ng/mL	.3466	.18737	.5800	.64066	≤0.05
Insulin µU/ml	10.4367	3.41104	16.9138	5.93972	≤0.001
FBG mg/dl	91.3556	6.99726	123.1111	16.68726	≤0.001
GSH ng/ml	.1981	.1274	.1161	.1397	≤0.05

Table 2 shows the metabolic indicators and body mass index (BMI) that are correlated with sestrin2. Except for glutathione, which showed a strong positive link with sestrin2 in PCOS women, the data showed that plasma sestrin2 levels were strongly inversely related to all other variables.

Correlation		
Parameters (Patients)	**Sestrin2**	
	r	***P* value**
BMI kg/m2	-.461	.001
8-OHdG nmol/ml	-.465	.001
HOM-IR	-.535	.000
Testosterone ng/mL	-.550	.000
Insulin µU/ml	-.507	.000
FBG mg/dl	-.403	.006
GSH ng/ml	.621	.000

TABLE 2. Sestrin2 plasma level correlations with anthropometric and biochemical variables

Figure (3) shows that the area under the curve for sestrin2 was 0.831 (95% confidence interval of 0.895 (0.518 to 0.764)), p = 0.036, suggesting that it has good sensitivity for diagnosing patients compared with healthy women. This suggests that sestrin2 is a suitable biomarker for diagnosing PCOS. The results of the ROC curve analysis confirmed this.



Test Result Variable (s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Sestrin2	.831	.044	.000	.745	.916

FIGURE 1: ROC curve of sestrin2 for the diagnosis of PCOS

This study examined the association of sestrin2 for 8-OHdG and testosterone in women with PCOS. The findings demonstrated that concentrations of sestrin2 in PCOS women were markedly decreased compared to control women. The findings align with a recent study indicating a notable reduction in sestrin2 in the PCOS women relative to the healthy groups. This study's findings contradict several prior studies on various pathological conditions that report elevated plasma levels of sestrin2 in patients compared to healthy controls, including cancer, neurodegenerative disorders, cardiovascular diseases, and lung diseases, which demonstrated increased sestrin2 levels in patient plasma [7, 19]. The upregulation of sestrin2 in these disorders is proposed to safeguard against damage linked to diverse stress circumstances [20]. Plasma sestrin2 levels have been dramatically raised in asthma patients [21], potentially serving as a compensatory mechanism to counteract persistent hypoxia. Moreover, substantial elevations in serum sestrin2 levels have been observed in Alzheimer's disease, and Parkinson's disease Showing an inverse relationship with mental and cognitive disorders [22]. Sestrin2's protective role is a suitable pathway owing to its activity in antioxidant defense and autophagy [23]. The variance between the findings of this study and earlier research may stem from the differing clinical situations examined, which lead to different qualitative responses to Sestrin2 protein. Sestrin2 is believed to be crucial in the adaptation to stress by initiating and stimulating antioxidant responses through many pathways, including p53 [6, 7].

According [9], the Sestrin2 protein has been linked to oxidative stress and could serve as a biomarker for elevated oxidative stress in PCOS. It might have antioxidant properties because it activates the Nrf2 antioxidant pathway, which is involved in nuclear factor E2-related factor 2 [24, 25]. Among the findings of the current study is the presence of a significant negative correlation between Sestrin2 and 8-OHdG, the products of nuclear material damage by reactive oxygen species, and in contrast, a decrease in the antioxidant glutathione, which is positively and significantly correlated with Sestrin2. This confirms the vital role of Sestrin2 in regulating many vital cellular functions, including its role in oxidative stress. The compound 8-OH is the primary stable consequence of oxidative damage to DNA caused by reactive oxygen species (ROS), with increased quantities detected in body fluids and

tissues under inflammation conditions [26]. Findings indicated that, in comparison to the group of control, individuals suffering from PCOS exhibited markedly elevated levels of 8-OHdG, while anti-oxidants such as glutathione were dramatically diminished. Several contemporary diseases, such as as cancers, heart disease, and metabolic diseases, are associated with oxidative DNA damage, with 8-OHdG serving as a significant biomarker for evaluating this damage [12]. The significant relationship among Sestrin 2 and 8-OHdG, an indicator of DNA damage induced by ROS, has been reinforced by Sestrin 2's ability to diminish oxidative stress in individuals with PCOS. Considering the known significance of oxidative stress in the pathophysiology of PCOS, comprehending this relationship is essential for assessing and managing the condition, identifying appropriate therapies, and elucidating the underlying etiology of PCOS.

Recent studies indicate increased oxidative stress in women with PCOS, potentially worsening hormonal imbalances, inflammation, and insulin resistance [27]. The outcomes of the metabolic and hormonal factors are displayed in Table 1. The reduced levels of Sestrin2 in individuals with PCOS may suggest their capacity to regulate low antioxidants, as evidenced by the findings of the present investigation (Table 1). which is attributed to its function in mitigating the intense production of reactive oxygen species in these individuals, along with increased exposure to additional harmful stimuli.

A primary feature of PCOS is increased androgen levels. [15] contend that androgen excess is a primary characteristic of PCOS and may serve as a significant etiological element in the syndrome's development, with a prevalence of 60–80%. Furthermore, insulin resistance (IR) is recognized as a supplementary element in the etiology of PCOS. [15] contend that hyperandrogenism is a core characteristic of PCOS and may possibly be a significant causal cause of the condition. IR is a recognized contributor to the onset of PCOS [28, 29]. Table (1) demonstrates a significance elevation in testosterone and HOMA-IR values in patients with PCOS relative to the group of control . Oxidative stress was demonstrated to affect ovarian stages and cause hormonal imbalances, worsening the condition [28]. Oxidative stress significantly influences endocrine and biochemical changes in women with functional ovarian hyperandrogenism, closely linked to insulin resistance and testosterone levels [30]. Patients suffering from polycystic ovarian syndrome may have higher total levels of testosterone mostly due to oxidative stress, which may enhance the production of androgen through increasing the expression of enzymes involved in the production of testosterone in vitro [31]. The relationship among androgens, oxidative stress, and insulin resistance underscores the intricate pathophysiology of PCOS and stresses the necessity of comprehending the mechanisms governing these interactions. The potential correlation among Sestrin2, 8-OHdG, and testosterone levels in women with polycystic ovary syndrome (PCOs) is significant, particularly considering Sestrin2's established involvement in mitigating the heightened oxidative stress associated with hyperandrogenism in womens. [31]. Consequently, Sestrin2 levels may be modified in PCOS, as indicated by the study findings, which demonstrated a substantial negative connection with 8-OHdG and testosterone levels (Table 2). The correlation between Sestrin2 and oxidative stress indicators such 8-OHdG and androgen levels in women with PCOS highlights its potential as a biomarker for oxidative stress and metabolic dysfunction in this disorder. The study results indicated that Sestrin2 serves as a significant and sensitive biomarker for diagnosing PCOS in women, as evidenced by the ROC curve test (Table 3). In conclusion, further study is necessary to comprehensively elucidate the relationship between Sestrin2 and androgen excess as well as oxidative damage associated with PCOS, and to explore the potential therapeutic advantages of targeting Sestrin2 to ameliorate metabolic and reproductive complications linked to PCOS.

REFERENCES

1. R. Deswal, V. Narwal, A. Dang, and C. S. Pundir, "The prevalence of polycystic ovary syndrome: a brief systematic review," *Journal of human reproductive sciences,* vol. 13, no. 4, pp. 261-271, 2020.

2. R. Ziaei, Z. Shahshahan, H. Ghasemi-Tehrani, Z. Heidari, M. S. Nehls, and R. Ghiasvand, "Inulin-type fructans with different degrees of polymerization improve insulin resistance, metabolic parameters, and hormonal status in overweight and obese women with polycystic ovary syndrome: A randomized double-blind, placebo-controlled clinical trial," *Food Science & Nutrition,* vol. 12, no. 3, pp. 2016-2028, 2024.

3. C. G. Baptiste, M.-C. Battista, A. Trottier, and J.-P. Baillargeon, "Insulin and hyperandrogenism in women with polycystic ovary syndrome," *The Journal of steroid biochemistry and molecular biology,* vol. 122, no. 1-3, pp. 42-52, 2010.
4. C. Lu, Y. Jiang, W. Xu, and X. Bao, "Sestrin2: multifaceted functions, molecular basis, and its implications in liver diseases," *Cell death & disease,* vol. 14, no. 2, p. 160, 2023.
5. L.-X. Wang, X.-M. Zhu, and Y.-M. Yao, "Sestrin2: its potential role and regulatory mechanism in host immune response in diseases," *Frontiers in immunology,* vol. 10, p. 2797, 2019.
6. Y. Liu, M. Li, X. Du, Z. Huang, and N. Quan, "Sestrin 2, a potential star of antioxidant stress in cardiovascular diseases," *Free Radical Biology and Medicine,* vol. 163, pp. 56-68, 2021.
7. Chen, Y., Huang, T., Yu, Z., Yu, Q., Wang, Y., Hu, J. A., ... & Yang, G. (2022). The functions and roles of sestrins in regulating human diseases. *Cellular & Molecular Biology Letters*, vol. 27, pp. 1-24, 2022.
8. G. Zainal, "Investigation of antioxidant markers in diabetic patients," *Archives of Razi Institute,* vol. 76, no. 5, p. 1453, 2021.
9. A. Bestel, B. Elmas, O. S. Günkaya, M. Bestel, and P. Y. Bahat, "Could sestrin protein in serum be a new marker of oxidative stress in patients with polycystic ovary syndrome?," *Gynecological Endocrinology,* vol. 38, no. 12, pp. 1109-1113, 2022.
10. H. Sova, U. Puistola, L. Morin-Papunen, and P. Karihtala, "Metformin decreases serum 8-hydroxy-2'-deoxyguanosine levels in polycystic ovary syndrome," *Fertility and sterility,* vol. 99, no. 2, pp. 593-598, 2013.
11. Cooke, M. S., Evans, M. D., Dove, R., Rozalski, R., Gackowski, D., Siomek, A., ... & Olinski, R. (2005). DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. " *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* vol. 574, no. 1-2, pp. 58-66, 2005.
12. A. Di Minno *et al.*, "8-Hydroxy-2-deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature," *Antioxidants & redox signaling,* vol. 24, no. 10, pp. 548-555, 2016.
13. A. Abd-Alsalam, I. G. Zainal, and G. A. Taqa, "Estimation of protein oxidation parameters in patients with diabetic nephropathy," in *AIP Conference Proceedings*, 2022, vol. 2394, no. 1: AIP Publishing.
14. M. Graille, P. Wild, J.-J. Sauvain, M. Hemmendinger, I. Guseva Canu, and N. B. Hopf, "Urinary 8-OHdG as a biomarker for oxidative stress: a systematic literature review and meta-analysis," *International journal of molecular sciences,* vol. 21, no. 11, p. 3743, 2020.
15. K. Wang, Y. Li, and Y. Chen, "Androgen excess: a hallmark of polycystic ovary syndrome," *Frontiers in Endocrinology,* vol. 14, p. 1273542, 2023.
16. P. Kempegowda, E. Melson, K. N. Manolopoulos, W. Arlt, and M. W. O'Reilly, "Implicating androgen excess in propagating metabolic disease in polycystic ovary syndrome," *Therapeutic advances in endocrinology and metabolism,* vol. 11, p. 2042018820934319, 2020.
17. S. Siddiqui, S. Mateen, R. Ahmad, and S. Moin, "A brief insight into the etiology, genetics, and immunology of polycystic ovarian syndrome (PCOS)," *Journal of assisted reproduction and genetics,* vol. 39, no. 11, pp. 2439-2473, 2022.
18. R. Azziz *et al.*, "The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report," *Fertility and sterility,* vol. 91, no. 2, pp. 456-488, 2009.
19. N. Rai and S. Dey, "Protective response of Sestrin under stressful conditions in aging," *Ageing Research Reviews,* vol. 64, p. 101186, 2020.
20. Y. Kishimoto *et al.*, "Association between plasma sestrin2 levels and the presence and severity of coronary artery disease," *Disease Markers,* vol. 2020, no. 1, p. 7439574, 2020.
21. Y. Kang *et al.*, "Sestrin2 is involved in asthma: a case–control study," *Allergy, Asthma & Clinical Immunology,* vol. 15, pp. 1-6, 2019.
22. Kamalzadeh, L., Moghaddamnia, M., Malakouti, S. K., Rashedi, V., Bahrampour, S., Sharifi, N., ... & Shariati, B. (2019). Prevalence of dementia among older patients: a hospital-based study in Iran. American *American Journal of Alzheimer's Disease & Other Dementias®,* vol. 34, no. 7-8, pp. 500-506, 2019.
23. S.-D. Chen, J.-L. Yang, T.-K. Lin, and D.-I. Yang, "Emerging roles of sestrins in neurodegenerative diseases: counteracting oxidative stress and beyond," *Journal of clinical medicine,* vol. 8, no. 7, p. 1001, 2019.
24. Wang, M., Xu, Y., Liu, J., Ye, J., Yuan, W., Jiang, H., ... & Wan, J. (2018). Recent insights into the biological functions of sestrins in health and disease. *Cellular Physiology and Biochemistry*, vol. 43, no. 5, pp. 1731-1741, 2018.

25. V. Ngo and M. L. Duennwald, "Nrf2 and oxidative stress: A general overview of mechanisms and implications in human disease," *Antioxidants,* vol. 11, no. 12, p. 2345, 2022.
26. A. Goriuc, K.-A. Cojocaru, I. Luchian, R.-G. Ursu, O. Butnaru, and L. Foia, "Using 8-Hydroxy-2'-Deoxiguanosine (8-OHdG) as a Reliable Biomarker for Assessing Periodontal Disease Associated with Diabetes," *International Journal of Molecular Sciences,* vol. 25, no. 3, p. 1425, 2024.
27. P. Sengupta, S. Dutta, and M. F. Hassan, "Polycystic ovary syndrome (PCOS) and oxidative stress," *Journal of Integrated Science and Technology,* vol. 12, no. 3, pp. 752-752, 2024.
28. L. Rahmatnezhad, L. Moghaddam-Banaem, T. Behrouzi Lak, A. Shiva, and J. Rasuli, "Free androgen index (FAI)'s relations with oxidative stress and insulin resistance in polycystic ovary syndrome," *Scientific Reports,* vol. 13, no. 1, p. 5118, 2023.
29. H. Teede, A. Deeks, and L. Moran, "Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan," *BMC medicine,* vol. 8, pp. 1-10, 2010.
30. S. Suresh and T. Vijayakumar, "Correlations of insulin resistance and serum testosterone levels with LH: FSH ratio and oxidative stress in women with functional ovarian hyperandrogenism," *Indian Journal of Clinical Biochemistry,* vol. 30, pp. 345-350, 2015.
31. Zou, P., Yang, X., Wang, J., Li, Y., Yu, H., Zhang, Y., & Liu, G. (2016). Advances in characterisation and biological activities of chitosan and chitosan oligosaccharides.190, 1174-1181.," *Endocrine,* pp. 1-12, 2024.