Available online on 15.05.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Evaluation of Some Hormones Total Antioxidant Capacity and Malondialdehyde Levels in Polycystic Ovarian Syndrome Women attending the gynecology Clinic at Nnewi

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Article Info:

Article History:

Received 02 March 2024
Reviewed 07 April 2024
Accepted 29 April 2024
Published 15 May 2024

Cite this article as:

Ihim AC, Onyenekwe CC, Eze NN, Obi PC, Osakue N, Awalu JC, Ikwelle TA, Evaluation of Some Hormones Total Antioxidant Capacity and Malondialdehyde Levels in Polycystic Ovarian Syndrome Women attending the gynecology Clinic at Nnewi, Journal of Drug Delivery and Therapeutics. 2024; 14(5):108-112

DOI: http://dx.doi.org/10.22270/jddt.v14i5.6539

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Abstract

Polycystic ovarian syndrome (PCOS), marked by oxidative stress and hormonal imbalances, causes infertility, insulin resistance, and diverse health problems. It not only affects physical health but also can strain marriages and lead to divorce, posing a notable societal issue. The levels of hormones (Estradiol (E2), Dihydroepiandrosterone sulfate (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (PROG) Testosterone (TEST)), total antioxidant capacity (TAC) and Malondialdehyde (MDA) in PCOS Women attending the gynecology Clinic at Nnewi were investigated. This cross-sectional study had 45 participants with PCOS as a test group and 45 participants without PCOS as the control group between the ages of 18-50 recruited. All the biochemical parameters were determined by enzyme-linked immunoassay technique. Data was expressed as Mean ± standard deviation. The differences in parameters studied between the PCOS group (test) and the control group were evaluated using an independent t-test. Statistical significance was set at p-value < 0.05. Result showed significant higher differences in the mean serum levels of DHEA (87.40±65.90), LH (34.7±36.1), FSH (19.61±14.73) and Testosterone (3.04±1.36) in women with PCOS compared with the control (1.035±0.54),(20.76±18.1), (13.2±10.19), and (3.04±1.36)(p< 0.05) respectively. A higher significant difference exists in the mean serum MDA values of the test group (women with PCOS compared with the control group(p<0.05). This study concluded that oxidative stress and hormone imbalance occurred among participants with PCOS attending the gynecology clinic of Nnamdi Azikiwe University Teaching Hospital in Nnewi.

Keywords: PCOS, Estradiol, DHEA, luteinizing hormone, FSH, progesterone, Testosterone, TAC

INTRODUCTION

Oxidative stress has been implicated in the etiology of many diseases including Polycystic ovary syndrome (PCOS). PCOS is a heterogeneous endocrine disorder that impacts many women of reproductive age worldwide [1]. This syndrome is often associated with enlarged and dysfunctional ovaries, excess androgen levels, resistance to insulin, etc [2]. It is estimated that approximately every 1 in 10 women face PCOS before menopause and struggle with its complications [3]. Although the high ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) and increased frequency of gonadotropin-releasing hormone (GnRH) are known as the underlying causes of PCOS [4], the exact etiology and pathology have not been comprehensively well-known [4,5]. Evidence suggests the role of different external and internal factors, including insulin resistance (IR), hyperandrogenism (HA), Environmental factors, genetics, and epigenetics. In addition, it is worth mentioning that PCOS increases the risk of further complications like cardiovascular diseases [5,6], type 2 diabetes mellitus [5,6], metabolic syndrome [6], depression, and anxiety [7].

At a joint consensus meeting of the American Society for Reproductive Medicine and the European Society of Human Reproduction and Embryology (ASRM/ESHRE), also known as the Rotterdam criteria, a refined definition of PCOS was agreed on, namely, the presence of two out of three criteria: (i) oligo- and anovulation, (ii) hyperandrogenism (clinical and biochemical), and (iii) polycystic ovaries with the exclusion of other etiologies [8].

Conversely, the Androgen Excess Society task force recognized four key features of PCOS: (1) ovulatory and menstrual dysfunction, (2) hyperandrogenemia, (3) clinical features of hyperandrogenism, and (4) polycystic ovaries[9]. Among patients diagnosed with PCOS, 75% have been clinically evidenced with menstrual disturbances[9]. Around 60-80% of PCOS patients were observed to have elevated circulating androgen levels [9]. The clinical features of hyperandrogenism

seen clinically in PCOS patients are hirsutism, acne, and androgenic alopecia. Among these, the most common presenting complaint of PCOS patients is hirsutism followed by acne and androgenic alopecia[9]. Other features manifested in PCOS patients include the presence of polycystic ovaries: either 12 or more follicles measuring 2-9 mm in diameter or an increased ovarian volume of more than 10 cm3 detected by ultrasonography[10].

The problems of PCOS are not only related to cycle regulation, acne, and hirsutism but in fact, it has also been strongly linked to dyslipidemia[11] and cardiovascular disease[12]. A 24-hour blood pressure monitoring revealed that young women with PCOS demonstrated an increase in both mean and systolic blood pressure [13]. Moreover, postmenopausal women who suffer from PCOS are at increased risk of developing hypertension [14]. In another study conducted by [15], it was found that PCOS is also associated with insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. Females suffering from PCOS are also at high risk of having obstructive sleep apnea or sleep disturbances [16]. The lifetime risk of endometrial cancer that has been estimated in these women is 2.7 times more than that in women without this syndrome [17]. The prolonged unopposed estrogen production in the endometrium from chronic anovulation is the main cause of the development of endometrial cancer. Many studies have shown that women with PCOS face a lot of risks for infertility, and if they do conceive, a meta-analysis concluded that women with PCOS have increased risks of pregnancy complications such as gestational diabetes and preeclampsia with negative effects on neonatal outcomes[18]. Moreover, it is also reported in a few studies that there is a significantly increased risk of depressive disorders in these women, which could partly be explained by the comorbidities and physical changes[19,20]. Considering all the complications of the disease, it is very important to acknowledge the great impact it has on the patients physically, psychologically, and socially. Thus, it is very crucial to diagnose PCOS early to reduce the incidence of undesirable complications.

Despite the serious complications developed in women with PCOS, several studies show women had poor knowledge and minimal awareness of PCOS[21,23]. Most women in the study population are unaware of the complications associated with this disorder[21]. A study conducted among adolescent girls shows that the lack of awareness and a negative lifestyle attitude toward PCOS prevent them from taking any measures to improve their lifestyle behaviors[22]. Hence this study the evaluation of some hormones' total antioxidant capacity and malondialdehyde levels in polycystic ovarian syndrome women attending the gynecology Clinic at Nnewi was done to provide more information on the involvement of lipid peroxidation and hormone imbalance in this syndrome.

MATERIALS, METHODS AND RESULTS

The reagents and kits for the biochemical analysis were commercially obtained and the manufacturer's standard operating procedures were strictly observed. This cross-sectional study was conducted in Nnewi North, Anambra state, southeast of Nigeria.

Study participants

This study which lasted from January to June 2023 was carried out at the Gynaecology clinic of Nnamdi Azikiwe Teaching Hospital, Nnewi, Anambra State. Nnamdi Azikiwe University Teaching Hospital is a Premier Tertiary Healthcare Institution that offers accessible and affordable health solutions, medical training, and research. It has its functional accident and emergency services and provides a wide range of medical, surgical, diagnostics, outpatient, rehabilitative, and support services. Nnewi has an estimated population of 1,113,546 people with the city covering over 1,076.9 square miles (2,789 km2)(National Population Commission, 2019. This is a cross-sectional study design in which blood samples were collected from the participants once.

Sample size

The sample size was calculated using G*power software version 3.0.10 (Universitat Dusseldorf, Germany). Power analysis for the difference between two independent means (two groups) was conducted in G*power to determine a sufficient sample size using an alpha of 0.05, a power of 0.80, and a large effect size of 0.8. Based on these, the calculated total sample size of 42 has 80% power to detect a difference of 0.25 at a significance level of 0.05. A total number of 90 participants was recruited for this study to make room for possible attrition. This comprises 2 major groups. This hospital-based cross-sectional study had 45 participants with PCOS as a test group and 45 participants without PCOS or fibroid as the control group. Already diagnosed PCOS patients attending the Gynecology clinic of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State with or without fibroid between the ages of 18-50 were recruited for this study. PCOS patients not attending the Gynecology clinic of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State with or without fibroid between the ages of 18-50 were not recruited for this study. Patients with other chronic diseases aside from PCOS were also excluded from this study.

Sampling technique

A random sampling technique was used for recruiting the participants as they were available until the sample size was achieved. Participants were individuals who met inclusion criteria and consented to the study after the purpose of the study had been explained to them.

They were assured of the confidentiality of the information obtained from them during and after the study and were at liberty to quit the study at any time without it affecting their treatment.

Blood Sample Collection and Processing

A tourniquet was wrapped around the arm,3-4inches above the collection site(the superficial vein that lies within the elbow pit)The needle cap was removed and held in line with the vein, pulling the skin tight, and the required amount of the blood was collected by pulling the plunger of the syringe out slowly, the tourniquet removed, cotton wool was placed on the collection site and the needle was removed and the venous blood sampling, five milliliters (5ml) volume was collected from each participant using 5.0ml sterile disposable syringe and dispensed into 5ml plain sample containers all labeled with the participant's name and age while the glucose sample was conducted using a glucometer. The blood in the plain containers was spun for 5 minutes at 3000 revolutions per minute(rpm) after allowing the blood to clot for 30 minutes and the serum was separated from the red cells using a dry clean Pasteur pipette into a dry clean plain specimen container. The sample was stored at -20 degrees Celsius, the analysis consisted of a hormonal assay technique using ELISA and antioxidant analysis using ORAC (the oxygen radical absorbance capacity) technique.

Collection of Samples

Five milliliters (5ml) of venous fasting blood sample were collected aseptically after 10-12 hours by venipuncture method from each subject. Two microliters (2µl) of the fasting blood sample were used for glucose estimation. The remaining blood sample was dispensed into the plain sample bottle,

allowed to clot, retract, and centrifuged at 4000rpm for 5 minutes the serum was then extracted into well labeled plain tube, and stored in aliquots of three at 4˚C until required for the determination of other biochemical parameters.

Laboratory methods

All the reagents were commercially obtained and the manufacturers' standard operating procedures were strictly observed

Estradiol, luteinizing hormone, follicle-stimulating hormone, progesterone, and testosterone, were determined by enzyme-linked immunosorbent assay using ACCUBIND ELISA kits as described by [23,24], also DHEA by the enzyme-linked immunosorbent assay as described by [25]. Lipid peroxidation (malondialdehyde) was evaluated as described by [26], while total antioxidant activity was determined as described by[27]

Statistical analysis

Statistical Package for Social Science (SPSS) (version 26.0) for Windows, SPSS Inc. Chicago, USA, was used to analyze the data. Data was expressed as Mean ± standard deviation (SD). The differences in parameters studied between the PCOS group (test) and the control groups were evaluated using an independent t-test. Statistical significance was set at p-value < 0.05.

No significant differences were observed in the mean serum levels of Estradiol (107.30±18.57) and progesterone (10.9±18.7) in the test group (women with PCOS) compared with the control (women without PCOS) (182.67±25.80) (12.55±17.9) (p>0.05). However significant higher differences exist in the mean serum levels of DHEA (87.40±65.90), LH (34.7±36.1), FSH (19.61±14.73) and Testosterone (3.04±1.36) in women with PCOS compared with the control (1.035±0.54), (20.76±18.1), (13.2±10.19), and (3.04±1.36) (p< 0.05) respectively.

Table 1: Comparison of the values of some hormones in women with PCOS and the control(mean ±SD)

Hormones	Control(N=45)	Test(PCOS)(N=45)	t-test	P-value
E2(pg/ml)	182.67±25.80	107.30±18.57	1.589	0.116
DHEA(ug/ml)	1.035±0.54	87.40±65.90	8.791	0.001
LH(IU/L)	20.76±18.1	34.7±36.1	2.310	0.023
FSH(Miu/ml)	13.2±10.19	19.61±14.73	2.397	0.019
PROG(ng/dl)	12.55±17.9	10.9±18.7	-425	0.672
TESTO(ng/dl)	2.24±.69	3.04±1.36	3.524	0.001

*Statistically significant at p<0.05.

keys, E2 =Estradiol, DHEA= Dihydroepiandrosterone sulphate, LH luteinizing hormone, FSH= follicle stimulating hormone, PROG= progesterone TESTO= Testosterone.

There is no significant difference observed in the mean serum levels of TAC of the test group (0.41784±0.41) compared with the control group (women without PCOS)(0.53960±0.46) (p>0.05), however a higher significant difference exists in the mean serum MDA values of the test group(women with PCOS)(39.08±28.77) compared with the control group(women without PCOS)(20.35±14.78) (p<0.05).

Table 2: Comparison of the values of some antioxidant levels in women with PCOS and the control(mean±sd)

Antioxidants	Control	Test	T-test	P-value
TAC(U/ml)	0.5396±0.46	0.41784±0.41	-1.326	0.747
MDA(nmol/ML)	20.35±14.78	39.08±28.77	-3.884	0.001

*Statistically significant at p<0.05. TAC=Total antioxidant capacity MDA=Malondialdehyde

DISCUSSION

Polycystic ovarian syndrome, characterized by ovarian dysfunction and hormonal imbalances, encompasses a range of related conditions that significantly contribute to infertility through disrupted ovulation [28]. Estradiol, the predominant form of estrogen in the body, plays a crucial role alongside progesterone to facilitate menstruation by ensuring an adequate hormonal balance. Most women diagnosed with PCOS often discover that their estrogen levels align with the typical range, from 25 to 75 pg/ml[29]. In this cross-sectional study, no significant differences were observed in the mean serum levels of estradiol and progesterone in the test subjects, compared to the control. A significantly higher difference in the mean serum levels of DHEA, LH, FSH, and testosterone was observed. The significantly higher difference in the serum DHEA agrees with the meta-analysis of case-control studies by[30] In PCOS, the brain areas controlling hormone regulation are less sensitive to progesterone, leading to irregular LH secretion [31] This significantly higher difference in the serum LH and FSH observed in this study, is in line with the work of[32]. This could be caused by altered sex steroid production, metabolic malfunction, or obesity. The mean serum levels of FSH in the test subjects were significantly higher, which agrees with a study by [33,32]. In healthy women, the LH to FSH ratio is usually between 1 and 2. However, in women with polycystic ovary syndrome, this ratio becomes reversed, potentially reaching as high as 2 or 3 [34] This imbalance can lead to anovulation and or menstrual irregularities associated with the syndrome. Numerous studies have demonstrated a notable elevation in oxidative stress (OS) markers among individuals diagnosed with polycystic ovary syndrome (PCOS) alongside conditions such as obesity, insulin resistance (IR),

cardiovascular disorders, and various diseases [35,36,37,38,39,40,41,42,43,44,45] The total antioxidant capacity (TAC) measures how well antioxidants can protect cells from damage caused by oxidative stress. People with PCOS tend to have lower TAC levels, which suggests they have more oxidative stress. When TAC levels are low, another measure called Total Oxidant Status (TOS) tends to be high [46] This research reveals that there is no significant difference in the mean serum TAC levels in the test subjects compared with the control, although the control group means is more in value however the mean value of Malondialdehyde (MDA)was significantly higher in the PCOS group compared with the control, indicating oxidative stress, and an increase in free radical generation among the PCOS participants hence it is also involved in the pathogenesis of PCOS, infertility, and hormone imbalance in this study. This observation is consistent with that of[40, 47] in the study titled Increased Prolidase Activity and oxidative stress in PCOS and supports [47]

CONCLUSION

This study concluded that oxidative stress and hormone imbalance occurred among participants with PCOS attending the gynecology clinic of Nnamdi Azikiwe University Teaching Hospital in

Nnewi.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Contributors

ACI, CCO, and PCO conceived and designed the research proposal. ON, PCO, NNE, and ACI performed sample collection, experiments, and data analysis. CCO, ACI, AJC, and NNE contributed to the final version of the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

The authors would like to pay their most profound gratitude to the management and staff of Nnamdi Azikiwe University Teaching Hospital Nnewi, and Reene Medical Diagnostic Laboratory, Awada, Anambra State, for all laboratory analyses of all biochemical parameters

Data availability

The data used to support the findings of this study are available from the corresponding author upon

reasonable request.

Funding:

No funding sources.

Conflict of interest:

None declared.

Ethical approval:

The study sought and obtained ethical approval from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital with reference no.
NAUTH/CS/66/VOL16/VER.3/87/2024/034

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