



A STUDY OF SEVERAL PHYSIOLOGICAL TRAITS IN A GROUP OF WOMEN WITH POLYCYSTIC OVARIES

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Abstract

Background: Polycystic ovary (PCOs) known as multifaceted disorder defined by a pathogenetic pathway that has yet to be fully understood. Besides hormonal imbalances, abnormalities in insulin signalling, and dysfunction of adipose tissue, oxidative stress, characterized by an imbalance caused by an overproduction of the oxidants within the confines limits antioxidant defences, has been widely implicated in the aetiology of the syndrome.

Objective: The purpose of this study to assessment of malondialdehyde (MDA), LH, FSH, vitamin C, testosterone in obese women and non-obese women with PCOs and compared them with control.

Material and methods: This study investigated a sample of 90 women, consisting of 60 patients and 30 controls, with ages ranging (24- 40) years. Samples were obtained from female individuals who were referred to Tikrit Hospital during the period from end of November 2022 to April 2023. The diagnosis was established by the medical staff through consultation and relied on the use of ultrasonography. The female participants in this research were categorised into three distinct groups. The initial cohort consisted of 30 women who were obese and diagnosed with PCOs. The second cohort comprised of 30 non-obese women who were diagnosed with polycystic ovary syndrome. Control group in the present study, consisted of healthy women, who were included to provide a basis for comparison. The ages of these women were similar to those of the women diagnosed with polycystic ovary.

Results: The present study show that the mean±SD of MDA were (0.388 ±0.043) in obese women that have PCOs, (0.202 ± 0.038) in non-obese women with PCOs, (0.113±0.023) in control, the differences were significantly at p-value 0.01. As for the mean±SD of vitamin C were (1.01±0.09) in obese, (0.983±0.121) in non-obese, (1.31±0.271) in control at p-vale 0.028. Furthermore, LH, FSH, testosterone was (8.11±1.88), (4.8±1.22) and (4.31±1.16) in obese women with PCOs respectively,





(7.95 ± 1.36), (4.53 ± 1.42) and (4.29 ± 1.03) in non-obese women with PCOS. (4.73 ± 1.61) (4.47 ± 1.39) and (1.92 ± 0.35) in control at p-value 0.02, 0.325, 0.01 respectively.

Conclusion: The study concluded that increase level of malondialdehyde in PCOs patient in compared with control, and the increases more in obese women than non-obese. While decrease vitamin C in both obese and non-obese women with PCOs in compare with control. Furthermore, no differences in FSH among groups. Whereas LH and testosterone increase in both obese and non-obese women with PCOs in compare with control.

Keywords: polycystic ovary, oxidative stress Malondialdehyde (MDA), LH, FSH, Testosterone.

Introduction

Polycystic ovary (PCOs) is a disease of significant community health concern, primarily affecting individuals in age of reproduction. It is characterised by various dysfunctions related to reproduction, metabolism, and psychological well-being. The estimated the prevalence of PCOS varies between 6% to as high as 26%⁽¹⁾. PCOS is characterized by an abnormal menstruation cycle, persistent anovulation, and hyperandrogenism. It is also linked to various health complications, such as infertility, resistance to insulin, obesity, atherosclerosis, and diabetes ⁽²⁾. The term "oxidative stress" is commonly employed to denote a state of an imbalance between its capacity to use antioxidants to protect against the damaging effects on free radicals and their production, resulting in DNA damage and/or cellular apoptosis. The cell's normal oxidation reaction can be disrupted, leading to the generation of free radicals and peroxides, which can have detrimental effects on cellular integrity. The consequences of oxidative stress are contingent upon the magnitude of alterations in DNA integrity, which can induce programmed cell death through apoptosis or necrosis ⁽³⁾. Malondialdehyde (MDA) is a byproduct that arises from the peroxidation of polyunsaturated fatty acids within cellular environments. The overproduction of malondialdehyde (MDA) is a result of an elevated presence of free radicals. The measurement of malondialdehyde (MDA) concentration is widely recognised as a reliable indicator of oxidative stress ⁽⁴⁾. The concentrations of MDA, stratified by age and BMI, exhibited a 47% increase in women diagnosed with polycystic ovary syndrome (PCOS) when compared to the control group. ⁽⁵⁾. Vitamin C is an excellent antioxidant. It restores fat-soluble vitamin E's antioxidant capabilities and reacts with aqueous peroxy radicals. especially those that are





around and inside the inner cellular membrane lipid peroxidation is decreased by antioxidant activity. Non-lipid nuclear material may be less susceptible to intracellular free radical assault ⁽⁶⁾. The menstrual cycle and ovarian function are regulated by vitamin C. Ascorbic acid excretion rises and falls before ovulation, then rises again once temperature rises. Ascorbic acid absorption in the pre-ovulatory ovary helps ovulation. Ascorbic acid levels in the corpus luteum are high and stimulate progesterone and oxytocin. Synthesis of collagen, needed for corpus luteum and follicle growth and post-ovulation ovary healing, may be caused by high ascorbic acid levels in the ovaries ⁽⁷⁾.

2. Materials and Methods

This study investigated a sample of 90 women, consisting of 60 patients and 30 controls, with ages ranging from 24 to 40 years. The samples were obtained from female individuals who were referred to Tikrit Hospital during the period from end of November 2022 to April 2023. The diagnosis was established by the medical staff through consultation and relied on the use of ultrasonography. The female participants in this research were categorised into three distinct groups. The initial cohort consisted of 30 women who were obese and a diagnosis with PCOS. The second cohort comprised of 30 non-obese women diagnosed with PCOs. The control group in this study consisted of healthy women n (30), who were included to provide a basis for comparison. The ages of these women were similar to those of the women diagnosed with polycystic ovary.

2.1 A collection of samples

A volume of approximately 5 ml of venous blood was obtained from each case using a sterile disposable syringe. The blood was subsequently transferred into gel tubes and left to coagulate at ambient temperature for a duration of 20 minutes. The samples were subjected to centrifugation at a speed of 3000 revolutions per minute (rpm) for a duration of 15 minutes. The resulting sera were subsequently extracted and distributed evenly into three Eppendorf tubes, with each tube containing 500 µl of sample. The tubes were then stored at a temperature of -30°C until they were utilised for the biochemical assay, which encompassed various parameters include: Malondialdehyde MDA, Vitamin C, LH, FSH, Testosterone.

The weight of an individual in kilogrammes divided by the square of their height in metres. The quantification of obesity can be achieved through the utilization of the Body Mass Index (BMI) classification system developed by the World Health Organisation (WHO) and the International Obesity Task Force.





2.2 Measurement of Serum malondialdehyde

Serum malondialdehyde level (MDA) was measured spectrophotometrically by UV-VIS spectrophotometer by the thiobarbituric acid method. The method was performed as follows: 200 μl of serum samples were taken into each tube. 800 μl phosphate buffer, 25 μl BHT solution, and 500 μl 30% TCA were added. The tubes were mixed in the vortex, capped, and kept in an ice bath for 2 hours. The tubes were readied to room temperature. The tubes were then capped and centrifuged at 2000 rpm for 15 min. 1 ml of the supernatant obtained from the centrifuge was transferred to other tubes. 75 μl EDTA and 25 μl TBA were added to 1 ml of the filtrates. The tubes were mixed in the vortex and kept in a hot water bath for 15 minutes (70°C). Then, they were brought to room temperature, and their absorbance was read on UV/Vis spectrophotometer at 532 nm⁽⁸⁾.

Measurement of Serum Vitamin C (ascorbic acid) concentrations

The concentration of vitamin C was determined by using the method described by Subash-Babu *et al*⁽⁸⁾. Precipitation of 100 μL of the samples was done with 250 μL of 5% ice-cold tricarboxylic acid. The precipitated sample was centrifuged at 6 500 rpm for 20 min by using tabletop centrifuge. The mixture of one-tenth of 1.0 mL of supernatant with 0.2 mL of 2, 4 dinitrophenylhydrazine: thiourea: copper sulfate was done. The mixture was incubated for at 37°C for 3 h. Finally, 1.5 mL of ice-cold 65% H₂SO₄ was added and thoroughly mixed. For another 30 min, the solution was kept at room temperature and the absorbance was read at 530 nm by using spectrophotometer. The values of vitamin C were expressed as $\mu\text{g}/\text{mg}$ protein.

Measurement of Serum, LH, FSH, Testosterone by Enzyme-Linked Immunosorbent Assay (ELISA)

Procedures were to quantify the plasma levels of LH, FSH, Testosterone concentrations and were determined using a sandwich ELISA; LH and FSH ELISA Kit (Mybiosource, USA). While Testosterone measured by the DRG Testosterone ELISA Kit

Statistical analysis

The results of current study were analyzed statistically using the statistical program (SPSS) version (23) by testing the mean and standard deviation and using the Duncan test to determine the differences between the groups at the level of probability (0.05)⁽⁹⁾.



3. Results

The present study shows increase of MDA in obese women that have PCOs, from non-obese women with PCOs and control that were (0.38 ± 0.043 , 0.202 ± 0.038 , 0.113 ± 0.023) respectively in control, the differences were significantly at p-value 0.01. As shown in Figure 1

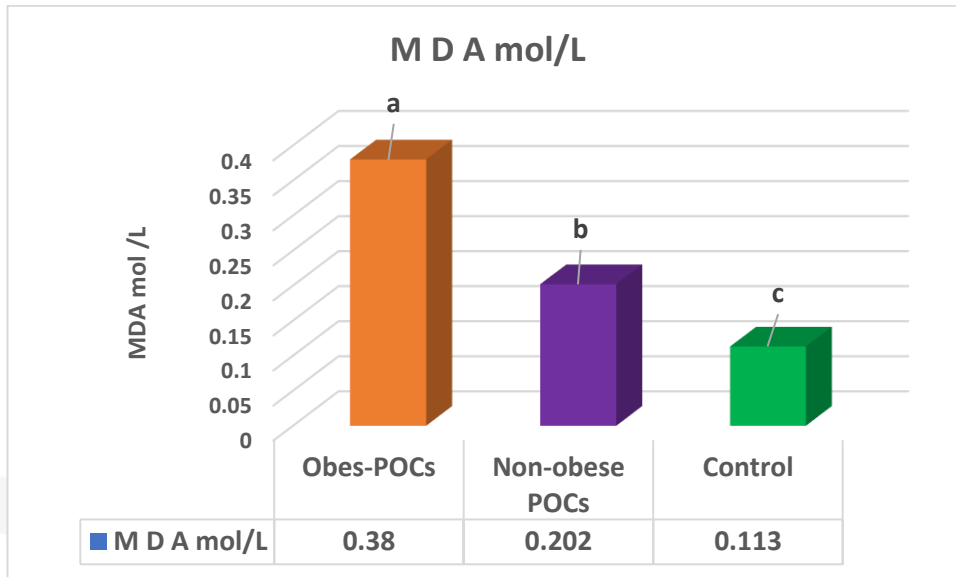


Figure 1: The level of MDA in study groups

The level of vitamin C decrease in obese and non-obese women that have PCOs were (1.01 ± 0.09 , 0.983 ± 0.121) respectively, in compared with control (1.31 ± 0.271) at p-vale 0.028. As shown in Figureo 2

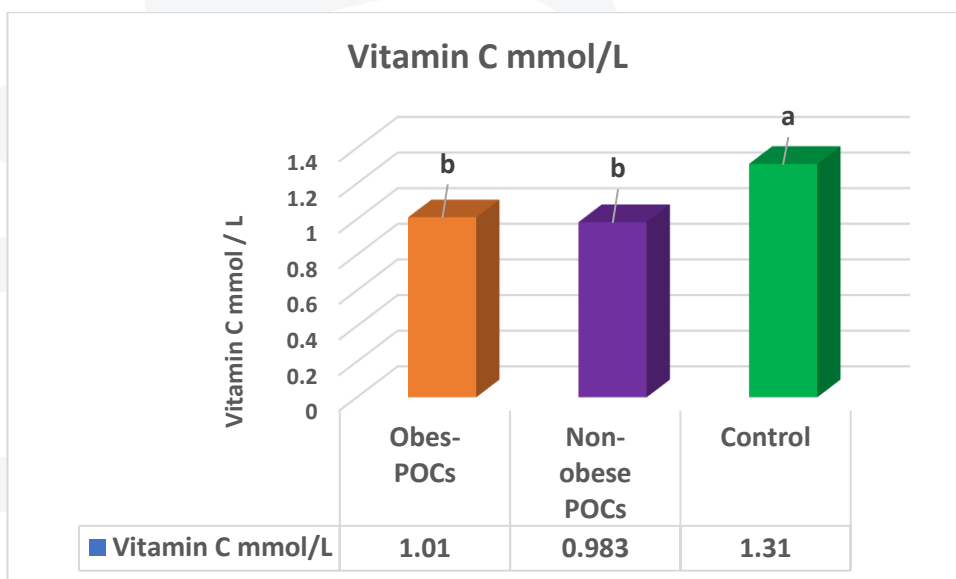


Figure 2: The level of vitamin C in study groups



The present study shows increase of LH in obese and non-obese women that have PCOs were (8.11±1.88, 7.95±1.36) respectively, in compared with control (4.73±1.61), at p-value 0.02. As shown in Figure 3

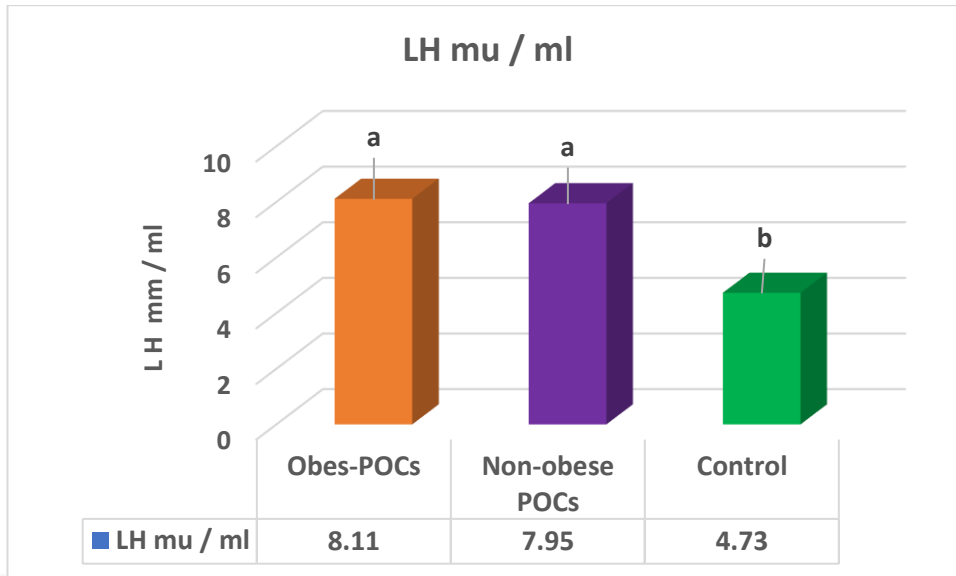


Figure 3: The level of LH in study groups

The present study shows no differences in the level of FSH in obese and non-obese women that have PCOs and with control that were (4.8±1.22, 4.53± 1.42, 4.47±1.39) respectively. at p-value 0.325. As shown in Figure 4

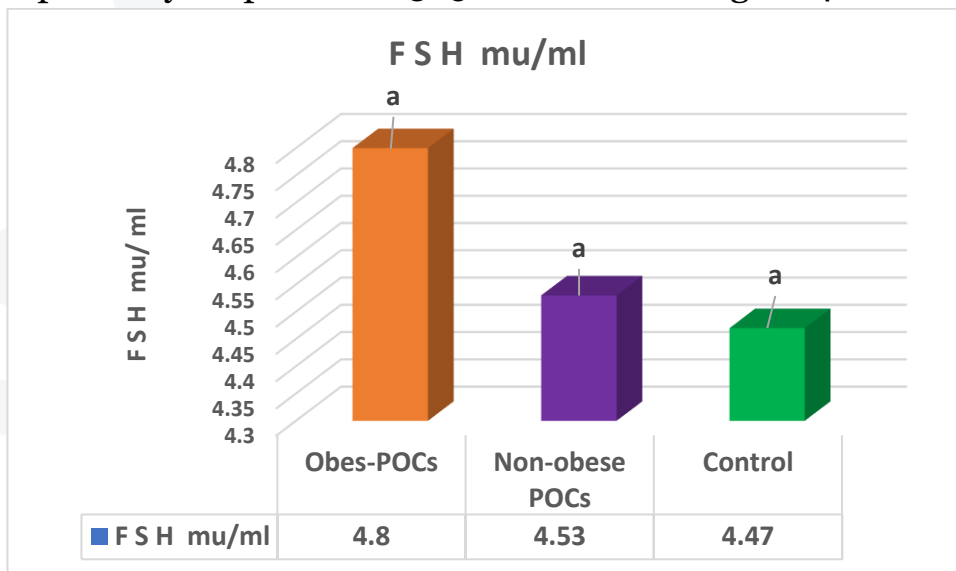


Figure 4: The level of FSH in study groups

The present study shows increase of Testosterone in obese and non-obese women that have PCOs were (4.31±1.16, 4.29±1.03) respectively, in compared with control (1.92±0.35), at p-value 0.01. As shown in Figure 5

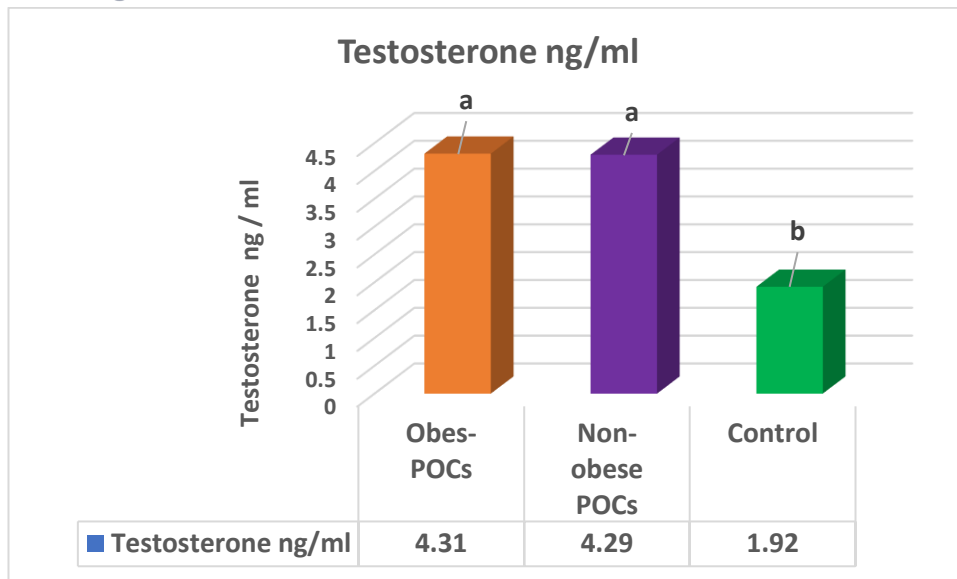


Figure 5: The level of testosterone in study groups

Discussion

This study shows increase level of MDA in Polycystic ovary significantly in compare with control groups, but MDA level in obese women with polycystic ovary more significantly than non-obese polycystic women at P-value 0.01. Our result agrees with TURAN⁽¹⁰⁾, UÇKAN⁽¹¹⁾ and SABUNCU⁽¹²⁾. MDA is a prevalent metabolic end-product that is produced during the process of lipid peroxidation. It serves a crucial function in the context of oxidative stress⁽¹³⁾. Therefore, it is possible that the increased levels of MDA can be ascribed to an amplified generation of reactive oxygen species (ROS), resulting in excessive oxidative harm within the individuals. The aforementioned oxygen species possess the capacity to undergo oxidation reactions with a range of vital biomolecules, including membrane lipids⁽¹⁴⁾. Elevated levels of (MDA) were observed in the serum of individuals diagnosed with polycystic ovary syndrome (PCOS) who were also classified as obese, as compared to non-obese counterparts with the same medical condition. The aforementioned observation can be ascribed to the correlation between obesity and the augmentation of oxidative stress⁽¹¹⁾. Other study done in India show increase level of MDA in obese women in compared with control⁽¹⁵⁾. In addition, the evaluation of serum MDA levels functions as a reliable indicator of the severity of damage caused by free radicals, thereby rendering it an invaluable instrument for assessing the adverse impacts of free radicals on cellular membranes⁽¹⁶⁾. The impact of hyperandrogenemia on The right balance of antioxidant and oxidant levels has been observed in women diagnosed with PCOs. The aforementioned disparity plays a substantial role in the emergence of oxidative stress in individuals diagnosed with PCOs⁽¹¹⁾. A recent study has



provided further evidence supporting the notion that hyperandrogenemia is a substantial contributor to oxidative stress's a prevalent in people with PCOs. Moreover, there have been suggestions indicating that metformin could potentially mitigate oxidative stress through the reduction of androgen levels. ⁽¹⁷⁾.

This study show decrease in vitamin C Level in obese and nonobese polycystic ovary. Statistically significant difference when compared to the control groups, with a p-value of 0.028. Our findings are consistent with MAHMUD ⁽¹⁸⁾. Other study show decrease in the level of vitamin c in polycystic ovary ⁽¹⁹⁾. Vitamin C is the main nonenzymatic water-soluble antioxidant and a cofactor in various metabolic processes. It is the first antioxidant used during free radical-induced lipid peroxidation. PCOS patients had considerably lower serum vitamin C levels than controls, which may be owing to vitamin C's role in free radical neutralisation⁽¹⁴⁾. Vitamin C should be studied as a treatment for PCOS-related ovarian morphology and anovulation. Vitamin C's effectiveness in treating PCOS ⁽²⁰⁾ Other study indicate that vitamin C depletes when MDA levels rise ⁽¹²⁾.

This study shows increase level of LH, testosterone in obese and non-obese women in Compared with control at p-vaule 0.02, 0.01 respectively, our study agrees with ⁽²¹⁾ GHAZI, while ⁽¹¹⁾ UÇKAN show the increase LH and testosterone in obese women rather than none obese. AS for FSH, our result show no differences between obese, non-obese and control and agree with ⁽¹¹⁾ UÇKAN.

Hyperandrogenism is the main PCOS indicator. 60%–80% of PCOS women have elevated testosterone ⁽²²⁾. PCOS is caused by hypothalamic GnRH secretion impairment. FSH and LH are released from the pituitary gland by GnRH. For the two menstrual stages, these hormones are needed. Egg formation is impossible without these hormones in PCOS. The egg stays in the follicle. This disrupts the cycle, causing primary or secondary amenorrhea ⁽²³⁾.

Androgen transforms peripherally to oestrogen. Second, it can regulate FSH release. Thus, dominant follicle development may be disrupted by follicle selection ⁽²⁴⁾. Elevated oestrogen levels increase pituitary sensitivity, which increases LH secretion and follicle growth and ovulation. Ovarian theca cells' androgen production is also at risk with elevated LH levels. It maintains hyperandrogenism. The reproductive system is at risk from this vicious spiral ⁽²⁵⁾. Weight loss is the most efficient way to restore ovulation and menstruation in obese PCOS patients ⁽²⁶⁾

5. Conclusions

The study concluded that increase level of malondialdehyde in PCOs patient in compared with control, and the increases more in obese women than non-obese.





While decrease vitamin C in both obese and non-obese women with PCOs in compare with control. Furthermore, no differences in FSH among groups. Whereas LH and testosterone increase in both obese and non-obese women with PCOs in compare with control.

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