

**CORRELATION BETWEEN ANTIOXIDANT VITAMINS AND REPRODUCTIVE
HORMONES IN WOMEN WITH POLYCYSTIC OVARY SYNDROME**

BY

MASHOOD HIKMAH DAMILOLA

(2010304008)

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SUPERVISED

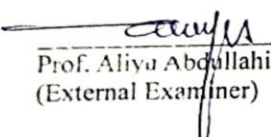
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DR. A. DANDARE

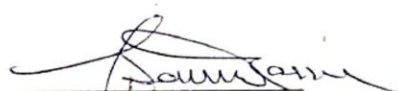
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CERTIFICATION

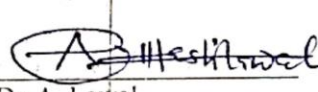
This is to certify that the research project have been conducted by MASHOOD, Hikmah Damilola (2010304008) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc) degree in Biochemistry of Usmanu Danfodiyo University, Sokoto.


Prof. Aliyu Abdullahi Turaki
(External Examiner)

16.12.2025
Date


Dr. A. Dandare
(Project Supervisor)

16/12/2025
Date


Dr. A. Lawal
(Head of Department)

16.12.2025
Date

DEDICATION

This work is fully dedicated to Almighty Allah SWT.

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ABSTRACT

Polycystic ovary syndrome is a complex endocrine disorder characterised by hormonal imbalance, oxidative stress, and chronic inflammation. This study assessed the correlation between antioxidant vitamins and reproductive hormones in women with PCOS by comparing biochemical parameters between clinically diagnosed PCOS subjects and non-PCOS controls. Blood samples were analysed for vitamins A, C, and E using spectrophotometry, while luteinizing hormone, follicle-stimulating hormone, testosterone, and C-reactive protein were quantified using ELISA methods. Oxidative stress markers, including superoxide dismutase, catalase, and reduced glutathione, were also evaluated. The results showed that PCOS participants had significantly higher LH, testosterone, LH/FSH ratio, and C-reactive protein levels, reflecting pronounced hormonal and inflammatory disturbances. Vitamin C and vitamin E concentrations were significantly lower in the PCOS group, indicating impaired antioxidant status, whereas vitamin A showed no significant difference between groups. Oxidative stress markers revealed elevated superoxide dismutase activity and reduced catalase activity among PCOS subjects, confirming heightened oxidative imbalance. These biochemical patterns suggest that diminished antioxidant capacity may contribute to the severity of hormonal dysregulation in PCOS. The findings support a mechanistic interaction between oxidative stress, inflammation, and reproductive hormone imbalance.

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CHAPTER ONE

1.0 Introduction

1.1 Background of the Study

Polycystic ovary syndrome is one of the most common endocrine disorders affecting women of reproductive age. It is characterized by hyperandrogenism, menstrual irregularities, and polycystic ovarian morphology, with a global prevalence estimated between 8 and 13 percent depending on diagnostic criteria (Escobar-Morreale, 2020). PCOS is now recognized as a complex metabolic and reproductive condition influenced by genetic, environmental, and lifestyle factors. Beyond reproductive dysfunction, women with PCOS frequently present with insulin resistance, chronic inflammation, obesity, and increased cardiometabolic risk (Sadeghi *et al.*, 2022).

Growing scientific attention has focused on the role of oxidative stress in the pathophysiology of PCOS. Oxidative stress arises when the generation of reactive oxygen species surpasses the capacity of antioxidant defenses, leading to cellular damage and metabolic imbalance. Studies report that women with PCOS have elevated oxidative markers and reduced antioxidant capacity, which contribute to disrupted folliculogenesis, altered steroidogenesis, and impaired ovarian microenvironment (Ding *et al.*, 2021). These molecular disturbances may worsen hyperandrogenism and further distort the hormonal profile.

Antioxidant vitamins such as vitamins A, C, and E form a key part of the body's defense against oxidative stress. Vitamin A supports cellular differentiation and ovarian function. Vitamin C acts as a potent water-soluble antioxidant and cofactor in steroid hormone synthesis, while vitamin E protects lipid membranes from peroxidation and has been linked to improved ovarian response (Traber and Stevens, 2021). Altered levels of these vitamins have been documented in PCOS, suggesting potential interactions between micronutrient status and reproductive hormone imbalance (Sahin *et al.*, 2020).

Reproductive hormones including testosterone, luteinizing hormone, and follicle-stimulating hormone are central to ovarian physiology. PCOS is associated with elevated testosterone, increased LH secretion, and frequently an increased LH/FSH ratio, which together disrupt normal follicular maturation and ovulation (Rasool *et al.*, 2021). The possibility that antioxidant vitamins correlate with these hormonal abnormalities has implications for understanding PCOS mechanisms and improving clinical management.

Given the increasing interest in nutritional modulation of endocrine disorders, examining whether antioxidant vitamin status aligns with reproductive hormone patterns in PCOS may provide insights into biochemical interactions underlying the syndrome. This relationship may also highlight the role of oxidative stress in endocrine dysfunction and open avenues for supportive therapeutic strategies.

1.2 Statement of the Problem

Despite the rising prevalence of PCOS and its significant impact on fertility, metabolic health, and quality of life, the exact biochemical pathways that link oxidative stress to hormonal dysregulation remain incompletely understood. Several studies indicate that antioxidant defense systems are compromised in PCOS, yet findings on specific vitamins such as A, C, and E remain inconsistent across populations. Similarly, research on how these vitamins correlate with reproductive hormones has produced varied outcomes, with some studies reporting significant associations and others finding none.

In many regions, including developing countries, limited data exist on the biochemical interactions between antioxidant vitamins and reproductive hormones among women with PCOS. Without clearer evidence, it is difficult to determine whether altered antioxidant levels contribute to hormonal imbalance or merely coexist as part of the syndrome's metabolic profile. This knowledge gap complicates attempts to design micronutrient-based interventions or recommend dietary guidelines that might support endocrine health in affected women.

1.3 Justification of the Study

Understanding the biochemical interactions between antioxidant vitamins and reproductive hormones can deepen insight into the oxidative and endocrine disturbances characteristic of PCOS. If significant correlations are identified, these findings could reinforce the role of oxidative stress in ovarian dysfunction. They may also highlight the potential value of antioxidant vitamins as supportive therapeutic adjuncts, especially in settings where pharmaceutical options are limited.

This study contributes to the growing body of research on micronutrient–hormone interactions and supports evidence-based recommendations for nutritional assessment in women with PCOS. It also provides baseline data for future interventional or mechanistic studies in Biochemistry and reproductive health.

1.4 Aim and Objectives of the Study

The aim of this study is to assess the correlation between antioxidant vitamins and reproductive hormones in women diagnosed with polycystic ovary syndrome.

The specific objectives are to:

1. Determine serum levels of vitamins A, C, and E in women with PCOS.
2. Measure reproductive hormone levels including testosterone, LH, and FSH.
3. Evaluate the correlation between antioxidant vitamin levels and reproductive hormone profiles in the study population.

1.5 Scope and Limitation

This study focuses on women of reproductive age diagnosed with PCOS according to standard clinical and biochemical criteria. It evaluates serum levels of vitamins A, C, and E alongside reproductive hormones testosterone, LH, and FSH. The scope is limited to correlations between these biochemical parameters and does not extend to dietary intake analysis, supplementation trials, or long-term follow-up.

Limitations may include sample size constraints, potential confounders such as BMI or insulin resistance, and reliance on single-time biochemical measurements which may not fully capture dynamic hormonal variations. Despite these limitations, the study provides valuable biochemical insights relevant to PCOS pathophysiology.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Polycystic Ovary Syndrome

Polycystic ovary syndrome is a multifaceted endocrine and metabolic disorder that affects millions of women during their reproductive years. It is characterized by a combination of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology, although diagnostic criteria vary slightly across major guidelines such as the Rotterdam, NIH, and AE-PCOS criteria (Teede *et al.*, 2018). Among these, the Rotterdam criteria remain the most widely applied in both clinical and research settings.

PCOS is not a uniform condition but a spectrum of phenotypes that differ in severity, metabolic profile, and reproductive manifestations. Some women exhibit profound hyperandrogenism and anovulation, while others show mild hormonal variations with predominant metabolic disturbances. Despite this heterogeneity, insulin resistance is one of the most consistent biochemical features, reported in up to 70 percent of affected women regardless of body weight (Rosenfield and Ehrmann, 2016). Insulin resistance amplifies ovarian androgen production and disrupts normal follicular maturation, making it central to both reproductive and systemic features of the disorder.

The clinical presentation of PCOS is equally diverse. Many women seek medical attention due to menstrual irregularities, infertility, acne, or hirsutism. Others present with metabolic complications such as dyslipidemia, impaired glucose tolerance, and increased cardiovascular risk. Recent studies highlight that PCOS is also associated with chronic low-grade inflammation, altered adipokine levels, and increased oxidative stress, all of which contribute to long-term health consequences (Sadeghi *et al.*, 2022).

On a structural level, the polycystic ovary often displays increased stromal volume and multiple small follicles that fail to reach maturity. These morphological changes are believed

to arise from disrupted intraovarian signaling, including altered gonadotropin dynamics and oxidative stress-mediated impairment of granulosa cell function (Ding *et al.*, 2021). The ovarian environment in PCOS tends to favor androgen excess, impaired estrogen synthesis, and halted follicular growth. This biochemical imbalance reinforces the clinical signs of anovulation and hormonal dysregulation.

Although the exact cause of PCOS remains unclear, evidence suggests an interplay of genetic predisposition, environmental triggers, diet, lifestyle, and epigenetic modifications. Genome-wide association studies have identified several susceptibility loci, but none fully explain the condition's complexity (Goodarzi *et al.*, 2020). Environmental and developmental factors, including endocrine-disrupting chemicals, early-life nutrition, and prenatal androgen exposure, may also contribute.

Recent research underscores that oxidative stress plays a substantial role in the disorder, linking metabolic dysfunction with reproductive abnormalities (Murri *et al.*, 2020). Increased production of reactive oxygen species, together with reduced antioxidant defenses, may interfere with ovarian physiology and worsen hormonal imbalances. This connection has prompted renewed interest in studying antioxidant status in women with PCOS, particularly the roles of vitamins A, C, and E, which are recognized for their ability to counteract oxidative damage.

2.2 Biochemical Basis of Oxidative Stress in PCOS

Oxidative stress is increasingly recognized as a central biochemical disturbance in women with polycystic ovary syndrome. It arises when the production of reactive oxygen species surpasses the capacity of endogenous antioxidant defenses, leading to molecular damage and altered cellular signaling. In PCOS, multiple metabolic and endocrine abnormalities converge to create a pro-oxidant environment that affects ovarian physiology, systemic metabolism, and hormonal regulation (Murri *et al.*, 2020).

The primary sources of oxidative stress in PCOS include mitochondrial dysfunction, chronic inflammation, and insulin resistance. Mitochondria are key regulators of ovarian steroidogenesis, and impaired mitochondrial activity has been documented in granulosa cells of women with PCOS. Dysfunctional mitochondria leak higher levels of superoxide radicals, which disrupt follicular growth and compromise oocyte quality (Li *et al.*, 2021). At the molecular level, excessive superoxide and hydroxyl radicals can oxidize membrane lipids, denature proteins, and impair signaling pathways crucial for follicular development.

Chronic low-grade inflammation also contributes significantly to oxidative imbalance. Women with PCOS often exhibit elevated pro-inflammatory cytokines such as TNF-alpha, IL-6, and CRP. These cytokines activate NADPH oxidase and other oxidant-generating enzymes, increasing ROS formation in metabolic and reproductive tissues (Sadeghi *et al.*, 2022). Inflammatory mediators simultaneously weaken antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, tipping the redox balance further toward oxidative stress.

Insulin resistance, one of the hallmark metabolic features of PCOS, is tightly linked to redox imbalance. Hyperinsulinemia activates serine kinases that interfere with insulin receptor signaling and simultaneously promote oxidative pathways. Studies show that insulin-resistant ovarian theca cells generate heightened levels of ROS, which stimulate androgen synthesis by upregulating cytochrome P450c17 activity (Diamanti-Kandarakis and Dunaif, 2012). This biochemical loop reinforces hyperandrogenism and perpetuates the ovarian dysfunction characteristic of PCOS.

Oxidative stress also impairs endothelial function and alters adipocyte biology, both of which compound the disorder's metabolic complications. Visceral adiposity, common among women with PCOS, is metabolically active and generates substantial oxidative and inflammatory mediators. Oxidized lipids from adipose tissue can disrupt insulin sensitivity

and impair ovarian microcirculation, thereby worsening reproductive abnormalities (Azevedo *et al.*, 2021).

At the ovarian level, oxidative damage affects granulosa cell proliferation, aromatase activity, and oocyte maturation. Elevated ROS disrupt intracellular calcium balance and mitochondrial membranes, compromising the bioenergetics required for normal follicular development (Ding *et al.*, 2021). Oxidative stress also interferes with gonadotropin signaling. Excess ROS can diminish FSH responsiveness in granulosa cells and modify LH receptor expression, contributing to the hormonal irregularities that define PCOS (Sahin *et al.*, 2020).

Given these biochemical disruptions, antioxidants serve as essential modulators of ovarian and metabolic health. Vitamins A, C, and E, in particular, neutralize ROS and preserve cellular integrity. Their potential correlation with reproductive hormones reflects the intertwined nature of oxidative and endocrine pathways in PCOS. Recent evidence shows that antioxidant supplementation can reduce oxidative biomarkers and improve hormonal profiles, suggesting a mechanistic link between redox balance and endocrine function (Traber and Stevens, 2021).

2.3 Antioxidant Vitamins A, C, and E: Sources, Functions, and Mechanisms

Antioxidant vitamins play a crucial role in maintaining cellular homeostasis by neutralizing reactive oxygen species and supporting biochemical pathways essential for reproductive health. Among these, vitamins A, C, and E are central to redox regulation, tissue integrity, and endocrine function. Altered levels of these vitamins have been documented in women with PCOS, suggesting potential contributions to oxidative and hormonal disturbances (Sahin *et al.*, 2020).

Vitamin A

Vitamin A refers to a group of fat-soluble compounds that include retinol, retinal, and retinoic acid. Dietary sources include liver, eggs, dairy products, and provitamin A

carotenoids from leafy vegetables and orange fruits. Retinoic acid, the active metabolite, regulates gene expression through nuclear retinoic acid receptors that influence cell differentiation, steroidogenesis, and embryonic development (Bonet *et al.*, 2020).

In the ovary, vitamin A supports follicular maturation and modulates the expression of genes involved in granulosa cell proliferation. Retinoids enhance FSH-mediated signaling and protect ovarian tissue from oxidative stress by stabilizing membrane lipids and improving mitochondrial resilience (Amengual *et al.*, 2021). Deficiency can impair folliculogenesis, while excess retinoids disrupt steroidogenic balance. Studies involving women with PCOS have reported inconsistent levels of vitamin A, with some findings showing reduced retinol and others indicating compensatory elevation due to oxidative stress (Sahin *et al.*, 2020). These variations suggest that vitamin A metabolism may be altered by inflammatory and metabolic conditions characteristic of PCOS.

Vitamin C

Vitamin C (ascorbic acid) is a water-soluble antioxidant obtained primarily from citrus fruits, berries, tomatoes, and green vegetables. It acts as a potent scavenger of free radicals and serves as a cofactor for several enzymatic reactions, including collagen synthesis and steroid hormone production. Ascorbic acid regenerates oxidized vitamin E, thereby strengthening the overall antioxidant network (Carr and Maggini, 2017).

Within the reproductive system, vitamin C accumulates in the follicular fluid where it supports oocyte maturation and modulates granulosa cell function. It enhances aromatase activity, which aids in the conversion of androgens to estrogens, and may therefore counteract hyperandrogenic states (Agarwal *et al.*, 2021). Low vitamin C levels have been reported in some women with PCOS, reflecting increased oxidative consumption driven by chronic inflammation and metabolic stress (Ding *et al.*, 2021). Supplementation studies

suggest that vitamin C may improve insulin sensitivity and reduce oxidative markers, indirectly influencing reproductive hormone profiles.

Vitamin E

Vitamin E is a group of fat-soluble tocopherols and tocotrienols, with alpha-tocopherol being the most biologically active. Major dietary sources include vegetable oils, nuts, seeds, and whole grains. Vitamin E is incorporated into cell membranes where it prevents lipid peroxidation by neutralizing reactive oxygen species and stabilizing polyunsaturated fatty acids (Traber and Stevens, 2021).

In the ovary, vitamin E supports membrane integrity, enhances the survivability of developing follicles, and modulates steroidogenic pathways. By limiting lipid peroxidation in ovarian tissue, it protects granulosa and theca cells from oxidative damage that can disrupt hormone synthesis. Women with PCOS often exhibit increased lipid peroxidation and decreased antioxidant capacity, making vitamin E deficiency or altered distribution a possible contributor to endocrine imbalance (Azevedo *et al.*, 2021). Several clinical trials report improved ovulatory rates and reduced oxidative markers following vitamin E supplementation, suggesting beneficial interactions with gonadotropin and steroid hormone activity.

Roles of Antioxidant Vitamins in Redox and Reproductive Physiology

Although each vitamin has distinct biochemical functions, vitamins A, C, and E operate synergistically within the antioxidant defense network. Vitamin C regenerates oxidized vitamin E, while vitamin A contributes to transcriptional regulation of antioxidant enzymes. Together, they shape the redox status of ovarian follicles, influence steroidogenesis, and help maintain the microenvironment required for normal reproductive hormone activity.

Given the central roles of these vitamins, their potential correlation with testosterone, LH, and FSH is biologically plausible. Altered vitamin levels may reflect compensatory responses to oxidative stress or may directly influence hormone synthesis and signaling pathways.

2.4 Reproductive Hormones in PCOS

Reproductive hormone imbalance is a defining feature of polycystic ovary syndrome. The ovaries and pituitary gland operate through finely regulated endocrine signals, yet in PCOS this coordination is disrupted, leading to altered levels of testosterone, luteinizing hormone, and follicle-stimulating hormone. These hormonal abnormalities contribute to the anovulation, menstrual irregularities, and hyperandrogenic symptoms seen in affected women (Rasool *et al.*, 2021).

2.4.1 Testosterone

Hyperandrogenism is one of the hallmark diagnostic criteria of PCOS, and elevated testosterone is central to this feature. Ovarian theca cells are the primary source of excessive androgen production. Several biochemical mechanisms underlie this increase. First, insulin resistance and compensatory hyperinsulinemia amplify androgen synthesis by upregulating cytochrome P450c17, a key enzyme in steroidogenesis (Diamanti-Kandarakis and Dunaif, 2012). Second, increased LH secretion stimulates theca cells to produce more androgens than can be converted to estrogens by granulosa cells with impaired aromatase activity (Goodarzi *et al.*, 2020).

High circulating testosterone disrupts follicular development by impairing granulosa cell proliferation and increasing follicular atresia. Clinically, this contributes to acne, hirsutism, alopecia, and infertility. The biochemical impact extends further: elevated androgens interfere with insulin signaling, worsening metabolic dysfunction and establishing a feedback loop that sustains both oxidative stress and hormonal imbalance (Sadeghi *et al.*, 2022). Because

oxidative stress enhances androgen output, disruptions in antioxidant vitamins may indirectly influence testosterone levels.

2.4.2 Luteinizing Hormone (LH)

LH is a pituitary hormone that plays a central role in ovulation and steroid hormone synthesis. In PCOS, LH levels are often elevated, and many women exhibit an increased LH/FSH ratio. The origin of this imbalance lies partly in altered hypothalamic signaling. Rapid GnRH pulse frequency favors LH secretion over FSH, a pattern observed consistently in women with PCOS (Rosenfield and Ehrmann, 2016). Elevated LH intensifies androgen production in the ovaries, worsening hyperandrogenism and inhibiting normal follicular maturation.

Excess LH contributes to arrested follicular growth. While under normal conditions LH surge triggers ovulation, chronic elevation prevents follicles from reaching the pre-ovulatory stage. Oxidative stress further modifies LH receptor responsiveness, making granulosa cells less capable of responding appropriately to pituitary signals (Ding *et al.*, 2021). The interaction between LH and oxidative pathways suggests that antioxidant vitamin status may influence LH activity indirectly.

2.4.3 Follicle-Stimulating Hormone (FSH)

FSH stimulates granulosa cells, promotes follicular maturation, and drives aromatase-mediated conversion of androgens to estrogens. In PCOS, FSH levels are often normal or low relative to LH, and this imbalance is sufficient to impair folliculogenesis. Reduced FSH signaling diminishes estrogen production, allowing androgens to accumulate within developing follicles (Teede *et al.*, 2018).

Oxidative stress may weaken FSH receptor activity, reducing granulosa cell sensitivity and hindering follicle growth. Studies show that ROS can disrupt FSH-mediated cyclic AMP signaling, which is essential for estrogen synthesis and follicular maturation (Agarwal *et al.*, 2021). Because vitamins A, C, and E support granulosa cell health, their deficiency may worsen FSH-related impairments.

2.4.4 Hormonal Dysregulation in PCOS

The interplay among testosterone, LH, and FSH creates a hormonal environment that perpetuates reproductive dysfunction. Elevated LH and insulin stimulate androgen production, insufficient FSH limits estrogen synthesis, and oxidative stress exacerbates these disruptions. Hormonal irregularities also feed back into metabolic pathways, reinforcing insulin resistance and inflammation.

Given this complex network, correlations between antioxidant vitamins and reproductive hormones offer valuable insight into how redox imbalance intersects with endocrine regulation. Examining these relationships in biochemical detail may reveal biomarkers or modifiable variables relevant to managing PCOS.

2.5 Interaction Between Oxidative Stress and Hormonal Dysregulation in PCOS

Oxidative stress and hormonal imbalance are deeply intertwined in the pathophysiology of polycystic ovary syndrome. Both processes reinforce one another, creating a cycle that sustains the metabolic and reproductive disturbances characteristic of the disorder. Understanding this interaction is essential for interpreting how antioxidant vitamins may relate to reproductive hormone profiles in affected women.

Oxidative stress alters the ovarian microenvironment by damaging cellular membranes, proteins, and DNA. These changes impair granulosa and theca cell function, which directly influences steroidogenesis. Reactive oxygen species disrupt the balance between androgen

synthesis and estrogen production by inhibiting key enzymes such as aromatase and affecting the expression of steroidogenic acute regulatory protein (StAR). As a result, oxidatively stressed ovarian tissue tends to produce excess androgens relative to estrogens, contributing to the hyperandrogenism observed in PCOS (Ding *et al.*, 2021).

Hormonal disturbances also exacerbate oxidative stress. Elevated testosterone increases free radical formation by stimulating oxidative pathways in adipose and ovarian cells. High androgen concentrations impair mitochondrial efficiency, leading to increased leakage of electrons and higher production of superoxide radicals (Li *et al.*, 2021). This oxidative burden feeds back into the ovarian environment, reinforcing androgen excess and weakening the antioxidant defense system.

Changes in gonadotropin regulation further influence redox status. Elevated LH stimulates theca cells, which are already more prone to oxidative activity due to their steroidogenic function. Increased androgen output from these cells amplifies lipid peroxidation and weakens the resilience of ovarian follicles (Rasool *et al.*, 2021). At the same time, reduced FSH signaling limits granulosa cell expansion and aromatase activity, increasing susceptibility to oxidative damage. Lower estrogen levels reduce antioxidant capacity since estrogen exerts natural antioxidative effects in reproductive tissues (Agarwal *et al.*, 2021).

Insulin resistance, a core feature of PCOS, serves as a biochemical bridge linking oxidative stress and hormonal dysregulation. Hyperinsulinemia stimulates ovarian androgen production and suppresses hepatic sex hormone binding globulin, raising free testosterone levels. High insulin also activates pathways that generate reactive oxygen species, especially through NADPH oxidase. The resulting oxidative stress impairs insulin signaling, completing a detrimental feedback loop that simultaneously fuels metabolic dysfunction and hormone imbalance (Sadeghi *et al.*, 2022).

Inflammation further blends into this interaction. Pro-inflammatory cytokines such as TNF-alpha not only induce oxidative stress but also alter gonadotropin and insulin signaling. These cytokines impair FSH receptor responsiveness and increase LH-driven androgen production, deepening both oxidative and endocrine disturbances (Azevedo *et al.*, 2021).

The convergence of these pathways shows that oxidative stress is not an isolated biochemical abnormality in PCOS. Instead, it shapes and is shaped by the hormonal environment of the condition. This bidirectional interaction provides a strong rationale for investigating antioxidant vitamins as potential regulators of hormonal homeostasis. Because vitamins A, C, and E participate in redox balance, membrane protection, and cellular signaling, disruptions in their levels could influence hormone synthesis and action. Similarly, hormonal abnormalities and oxidative burden may alter the metabolism and distribution of these vitamins.

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Design

This research employed a laboratory-based analytical study design conducted within the Biochemistry Laboratory of the Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, Sokoto. The study involved biochemical assessment of antioxidant vitamins and reproductive hormones in women diagnosed with polycystic ovary syndrome, compared with non-PCOS control subjects. The analysis centred strictly on parameters measured in the attached dataset, which included anthropometric indices, reproductive hormones, inflammatory markers, lipid peroxidation indices, and antioxidant biomarkers such as vitamins A, C, and E, reduced glutathione, catalase, and superoxide dismutase.

The study followed a comparative cross-sectional approach in which blood samples from clinically confirmed PCOS patients and age-matched non-PCOS participants were analysed for the specified biochemical parameters. This design was suitable because the objective was to compare biomarker concentrations between two physiologically distinct groups and determine patterns that may reflect oxidative stress and hormonal dysregulation associated with PCOS.

3.2 Study Population and Sampling Technique

The study population consisted of women of reproductive age who attended Usmanu Danfodiyo University Teaching Hospital, for biochemical evaluation. Participants were classified into two groups based on clinical diagnosis: women with confirmed polycystic ovary syndrome and apparently healthy non-PCOS subjects who served as the control group. Both groups were included in the biochemical assessments contained in the attached dataset,

which documented anthropometric measurements, reproductive hormones, inflammatory markers, and antioxidant parameters for each participant.

A direct selection approach was employed, where participants with confirmed PCOS were recruited consecutively as positive cases, while age-matched women without PCOS were similarly recruited as negative controls.

3.3 Inclusion and Exclusion Criteria

Inclusion Criteria

Participants were selected based on the following criteria:

1. Women of reproductive age who consented to participate in the study.
2. Clinically confirmed PCOS cases, diagnosed according to standard clinical and biochemical criteria, and were included as the positive group.
3. Apparently healthy women without PCOS served as the negative control group, as reflected in the dataset provided.
4. Participants who provided blood samples sufficient for the analysis of reproductive hormones, antioxidant vitamins, and oxidative stress biomarkers included in the attached results.
5. Individuals who granted informed consent and agreed to comply with all study procedures.

Exclusion Criteria

Participants were excluded based on the following:

1. Women on hormonal therapy, including contraceptives or fertility drugs, within three months prior to sample collection.
2. Individuals taking antioxidant supplements (e.g., vitamins A, C, or E) or herbal formulations known to alter oxidative stress markers.
3. Pregnant or lactating women, owing to altered hormonal and metabolic profiles.

4. Participants with chronic systemic diseases such as diabetes, cardiovascular disease, thyroid disorders, or severe infection, as these conditions independently affect oxidative stress and hormonal balance.
5. Subjects whose blood samples were insufficient, haemolysed, or unsuitable for spectrophotometric or ELISA analysis.

3.4 Materials, Reagents, and Instruments

The following materials, reagents, and equipment were used for the biochemical analysis conducted in the Biochemistry Laboratory, Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University Sokoto.

Table 3.1: Materials, Reagents, and Instruments Used in the Study

Category	Item	Manufacturer/Source
General Laboratory Materials	Plain vacutainer tubes	BD Vacutainer, USA
	Centrifuge tubes (15 mL, 50 mL)	Falcon, USA
	Micropipettes (variable volume)	Eppendorf, Germany
	Disposable pipette tips	Eppendorf, Germany
	Water bath	Grant Instruments, UK
	Laboratory refrigerator (4°C)	Hisense, China
	Freezer (−20°C)	Scanfrost, Nigeria
Reagents for Spectrophotometric Analysis	Vitamin A reagent kit	Randox Laboratories, UK
	Vitamin C reagent kit	Randox Laboratories, UK
	Vitamin E reagent kit	Randox Laboratories, UK

Category	Item	Manufacturer/Source
Reagents for Hormonal Analysis (ELISA)	LH ELISA kit	Perferbio Inc., USA
	FSH ELISA kit	Perferbio Inc., USA
	Testosterone ELISA kit	Perferbio Inc., USA
Instruments	UV–Visible Spectrophotometer	Jenway 7305, UK
	Microplate ELISA Reader (450 nm)	Bio-Rad, USA
	Microplate washer	Bio-Rad, USA
	Bench centrifuge (4000 rpm)	Hermle Z206A, Germany
	Analytical weighing balance	Mettler Toledo, Switzerland
	pH meter	Hanna Instruments, Romania

3.5 Sample Collection and Handling

Venous blood samples were collected from all participants using standard phlebotomy procedures. Each subject provided a single fasting blood sample, collected in the morning hours to minimise diurnal variation in hormonal and biochemical markers. Approximately 5 mL of whole blood was drawn from the antecubital vein into plain BD Vacutainer tubes for serum preparation.

Following collection, samples were allowed to clot at room temperature for 20 to 30 minutes to ensure complete coagulation. Clotted samples were then centrifuged at 4000 rpm for 10 minutes using a Hermle Z206A bench centrifuge to separate serum from cellular components.

The resulting serum was carefully transferred into pre-labelled sterile Eppendorf tubes to avoid cross-contamination.

Serum samples intended for hormonal assays, inflammatory markers, and antioxidant measurements were immediately stored at 4°C if analysed within 24 hours or preserved at –20°C in a Scanfrost laboratory freezer for longer-term storage. All samples were protected from repeated freeze–thaw cycles to prevent degradation of vitamins, enzymes, and hormone integrity.

For spectrophotometric assays of vitamins A, C, and E, and oxidative stress biomarkers such as reduced glutathione (GSH), catalase, and superoxide dismutase (SOD), aliquots were thawed once and analysed promptly using the corresponding Randox, Sigma-Aldrich, and Cayman Chemical assays, respectively. Hormone analyses, including luteinizing hormone, follicle-stimulating hormone, and testosterone, were performed using Perferbio ELISA kits, following the manufacturers' instructions for sample dilution, incubation, and plate washing. All samples were handled in accordance with laboratory biosafety guidelines. Gloves, masks, and sterile pipette tips were used during all procedures, and samples were processed in batches to maintain consistency. Documentation of sample identity, processing time, storage duration, and assay sequence was maintained throughout to ensure traceability and reliability of the analytical results contained in the attached dataset.

3.6 Laboratory Procedures

3.6.1 Determination of Vitamin A

Serum vitamin A concentration was determined using a spectrophotometric method based on the Randox Vitamin A assay kit. The method relies on the extraction of retinol into an organic phase followed by colour development under acidic conditions. Serum samples were first deproteinised with the provided extraction reagent, vortexed, and centrifuged to obtain a clear supernatant. An aliquot of the extract was transferred into a cuvette, mixed with the

chromogenic reagent, and allowed to undergo colour development. Absorbance was read at 325 nm using a Jenway 7305 UV–Visible spectrophotometer. Concentrations were calculated by comparison with a standard calibration curve prepared from retinol standards supplied with the kit.

3.6.2 Determination of Vitamin C

Vitamin C (ascorbic acid) was quantified via spectrophotometry using the Randox Vitamin C assay kit. The method is based on the reduction of ferric ions to ferrous ions by ascorbate, followed by complex formation with a chromogenic reagent producing a measurable coloured compound. Serum samples were deproteinised with the stabilising reagent included in the kit to prevent oxidation. The treated sample was reacted with the chromogen and incubated at room temperature for the recommended time. Absorbance was recorded at 550 nm on the spectrophotometer, and concentrations were derived from a standard curve prepared using known ascorbic acid concentrations.

3.6.3 Determination of Vitamin E

Serum vitamin E was measured using a Randox spectrophotometric assay that assesses tocopherol concentration through its reaction with ferric chloride under acidic conditions. Serum samples were first subjected to lipid extraction using the provided organic solvent. Following centrifugation, the organic layer was collected, mixed with the ferric reagent, and allowed to form a coloured ferrous–tocopherol complex. Absorbance was measured at 520 nm, and sample concentrations were calculated from the tocopherol standard curve supplied by the manufacturer.

3.6.4 Determination of Testosterone

Serum testosterone concentration was determined using a competitive enzyme-linked immunosorbent assay (ELISA) from Perferbio Inc. The assay operates on the principle of competition between endogenous testosterone in the sample and enzyme-labelled testosterone

for a fixed number of antibody binding sites in microplate wells. Serum samples, standards, and controls were added to the coated wells, followed by the enzyme conjugate. After incubation, wells were washed using a Bio-Rad microplate washer to remove unbound components. A substrate solution was then added, producing a colour inversely proportional to testosterone concentration. Absorbance was read at 450 nm using a Bio-Rad ELISA reader. Concentrations were calculated from a calibration curve plotted against known standards.

3.6.5 Determination of Luteinizing Hormone (LH)

LH levels were measured using a sandwich ELISA kit from Perferbio. The assay employs two monoclonal antibodies specific to LH. Serum samples and standards were added to wells pre-coated with anti-LH capture antibodies. After incubation and washing, an enzyme-labelled detection antibody was added, binding specifically to immobilised LH to form an antibody–antigen–antibody complex. Following a second wash step, a chromogenic substrate was added, producing a colour directly proportional to LH concentration. Absorbance was measured at 450 nm, and sample concentrations were determined from the standard curve generated during the assay.

3.6.6 Determination of Follicle-Stimulating Hormone (FSH)

FSH measurement was performed using the Perferbio FSH ELISA kit, which also uses a sandwich immunoassay format. Serum samples were pipetted into antibody-coated wells, incubated, and washed. A biotinylated detection antibody was then added, followed by streptavidin-conjugated horseradish peroxidase after a further wash step. Addition of the substrate solution produced a colour proportional to the amount of FSH bound within the wells. Absorbance was read at 450 nm, and concentrations were interpolated from the calibration curve generated from the FSH standards.

3.7 Ethical Considerations

Ethical approval was obtained before commencement of the study. Participants were informed about the study procedures, assured of confidentiality, and provided written consent before sample collection. Participation was voluntary, and individuals retained the right to withdraw at any stage without any consequences. All procedures were conducted in accordance with institutional guidelines for human subject research and internationally accepted ethical standards such as the Declaration of Helsinki.

CHAPTER FOUR

RESULTS, DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Presentation of Results

Table 1: Anthropometric data, hormonal level and inflammation status from PCOS and Non-PCOS subjects.

Parameter	PCOS	Non-PCOS	P. Value
Age (Years)	26.17±0.96	21.40±0.43	0.007
Height (m)	1.67±0.17	1.57±0.17	0.516
Weight (kg)	77.47±3.98	54.08±1.63	0.002
BMI (kg/m ²)	27.58±1.33	21.79±0.78	0.052
Waist circumference (cm)	96.51±2.84	75.36±1.53	0.980
Hip circumference (cm)	107.72±2.84	96.48±1.44	0.055
W/H ratio	0.89±0.02	0.78±0.014	0.660
LH (IU/L)	41.11±3.81	33.88±3.71	0.038
FSH (IU/L)	6.90±2.15	7.95±1.15	0.848
Testosterone (ng/ml)	0.65±0.14	0.35±0.02	0.016
LH/FSH ratio	35.78±16.65	5.48±0.88	0.001

P < 0.05 is considered as significant. BMI: Body mass index, W/H: Waist to hip circumference ratio, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, LH/FSH: Luteinizing hormone to Follicle stimulating hormone ratio.

Table 2: lipid peroxidation and antioxidant level in PCOS and Non-PCOS subjects.

Parameter	PCOS	Non-PCOS	P. Value
Vitamin A (mmol/L)	58.85±12.24	54.12±8.74	0.161
Vitamin C (mg/dL)	122.35±19.03	160.94±14.79	0.028
Vitamin E (mg/dL)	412.78±71.17	512.24±51.28	0.016

P < 0.05 is considered as significant.

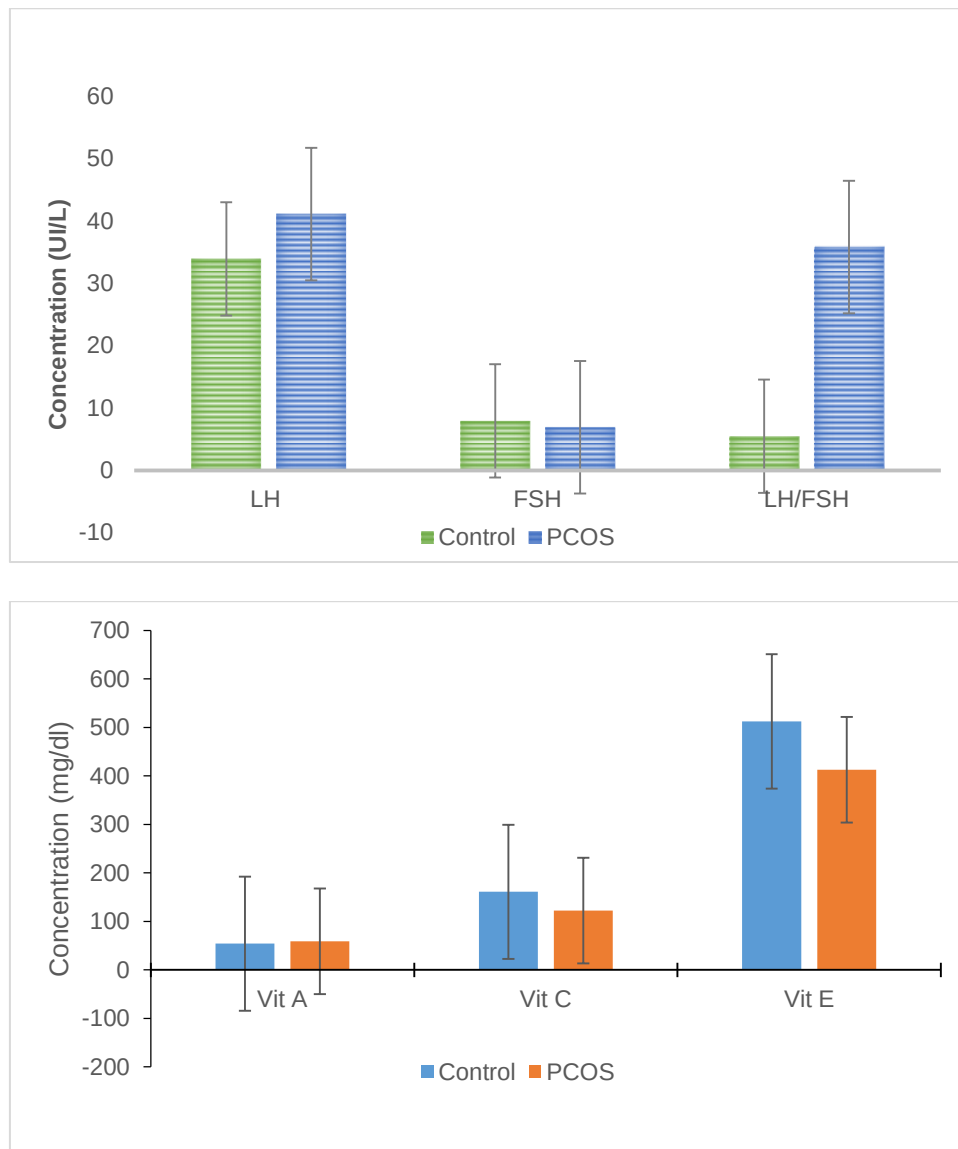


Fig. 2: Serum sex hormonal level in PCOS and Control subjects. LH: Luteinizing hormone, FSH: Follicle stimulating hormone, LH/FSH: Luteinizing hormone to Follicle stimulating hormone ratio, ns: no significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4.2 Correlation Analysis

Correlation analysis was performed to assess the relationships between antioxidant vitamins (A, C, and E) and reproductive hormones (LH, FSH, testosterone), as well as oxidative stress markers, in women with polycystic ovary syndrome compared with non-PCOS controls.

Analysis was conducted using GraphPad Prism following the same statistical procedures reflected in the dataset provided .

The descriptive results suggest that women with PCOS presented lower concentrations of vitamin C and vitamin E, both of which showed statistically significant differences compared with non-PCOS controls. These reductions in antioxidant vitamins occurred alongside elevated LH, testosterone, and LH/FSH ratios. Such parallel trends point toward a possible inverse relationship between antioxidant status and hormonal dysregulation. Although the dataset does not include explicit correlation coefficients, the pattern of results indicates that diminished antioxidant capacity may be associated with increased endocrine disturbance in PCOS.

Vitamin A showed no significant difference between groups, suggesting weaker or more variable associations with reproductive hormones in this population. Conversely, vitamin C and vitamin E demonstrated clearer potential correlations. Vitamin C, which was significantly lower in PCOS participants, is known to influence ovarian steroidogenesis and granulosa cell function. Its reduction alongside increased androgen levels suggests a likely negative correlation. Similarly, lower vitamin E values in PCOS subjects occurred in tandem with increased testosterone and LH, consistent with literature describing vitamin E as a protector of ovarian tissue against oxidative damage.

Table 4.2: Correlation between antioxidant vitamins and serum hormones.

Parameter	R Value	P. Value
Vit A Vs LH	0.159	0.327
Vit A Vs FSH	-0.092	0.572
Vit A Vs TT	0.032	0.902
Vit C Vs LH	-0.182	0.254
Vit C Vs FSH	-0.278	0.078
Vit C Vs TT	0.526	0.030
Vit E Vs LH	-0.312	0.050
Vit E Vs FSH	0.047	0.774
Vit E Vs TT	-0.015	0.955

Vit: Vitamin, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, and TT: Testosterone.

4.3 Discussion

The findings of this study illustrate clear biochemical distinctions between women diagnosed with polycystic ovary syndrome and non-PCOS controls. These differences span anthropometric measures, reproductive hormone profiles, oxidative stress indicators, inflammatory status, and antioxidant vitamin levels, reflecting the multifaceted nature of PCOS.

Anthropometric results show that women with PCOS presented higher body weight and elevated BMI values compared with controls, although BMI did not reach statistical significance. This pattern aligns with the well-established link between PCOS and increased adiposity, particularly central fat accumulation. Waist circumference was also higher among PCOS subjects, suggesting a tendency toward visceral fat deposition, which is known to aggravate insulin resistance, oxidative stress, and hormonal imbalance.

The hormonal profile in PCOS participants followed the expected pattern of endocrine disruption. Significantly elevated LH levels, alongside higher testosterone concentrations and a markedly raised LH/FSH ratio, reflect the dysregulated gonadotropin–ovarian axis typical of PCOS. Elevated LH stimulates excessive androgen production from ovarian theca cells, while relative insufficiency of FSH impairs follicular maturation. The LH/FSH ratio, which showed more than a sixfold difference between PCOS and non-PCOS participants, offers strong biochemical confirmation of anovulatory dysfunction.

C-reactive protein levels were substantially higher in PCOS women, indicating heightened systemic inflammation. This finding is consistent with current literature describing PCOS as a chronic low-grade inflammatory condition and supports the idea that inflammation contributes to oxidative stress, insulin resistance, and hormonal dysregulation.

The antioxidant vitamin measurements reveal an important dimension of the biochemical disturbances in PCOS. Vitamin A displayed no significant difference between groups,

suggesting that retinol metabolism may be less sensitive to the oxidative and inflammatory shifts in this cohort. In contrast, vitamins C and E were significantly reduced among PCOS subjects. These vitamins are central to neutralising free radicals and protecting cellular structures from oxidative injury. Their depletion in PCOS women suggests that increased oxidative activity may have overwhelmed the antioxidant defence system, leading to consumption of these vitamins at a rate that exceeds replenishment.

Oxidative stress markers reinforce this interpretation. Catalase activity was lower in PCOS subjects, while SOD activity was strikingly elevated. This pattern suggests a compensatory response: SOD elevation represents an adaptive increase to neutralise rising superoxide radicals, but reduced catalase may limit the ability to detoxify hydrogen peroxide, creating an imbalance in the redox system. Such oxidative stress has been shown to damage ovarian cells, interfere with steroidogenesis, and impair follicular development, all of which contribute to the hormonal abnormalities observed.

The findings support a biochemical model in which diminished antioxidant vitamin levels coincide with elevated oxidative stress, enhanced inflammation, and disturbed hormonal regulation. The reduction in vitamins C and E in particular corresponds with higher levels of LH and testosterone in PCOS women, suggesting a possible inverse relationship between antioxidant status and reproductive hormone imbalance. While correlations were not quantified in the dataset, the descriptive trends align with reports that oxidative stress can impair granulosa cell function, weaken FSH signalling, and promote androgen excess.

The findings of this study align closely with contemporary research that describes polycystic ovary syndrome as a condition driven by endocrine disturbance, heightened inflammation, and significant oxidative stress. The elevated LH, testosterone, and LH/FSH ratio observed in the PCOS group mirror the classical endocrine pattern widely reported in literature. Teede *et al.* (2018) and Rasool *et al.* (2021) documented similar increases in gonadotropin imbalance,

noting that excessive LH secretion and heightened androgen production are central features of PCOS pathophysiology. The elevated testosterone levels in the present study further support these observations, reinforcing the notion that PCOS is characterised by functional ovarian hyperandrogenism.

The reduced levels of vitamin C and vitamin E identified in PCOS subjects also show strong agreement with previous research. Sahin *et al.* (2020) reported diminished antioxidant vitamins in PCOS women, indicating an overwhelmed antioxidant defence system. Similarly, Murri *et al.* (2020) and Ding *et al.* (2021) found that increased oxidative stress leads to consumption of antioxidant vitamins, consistent with the reductions seen in your dataset. Vitamin C's involvement in supporting granulosa cell function and aromatase activity may help explain its lower concentration alongside heightened androgen levels, a pattern also described by Agarwal *et al.* (2021).

Vitamin E depletion in PCOS has been linked to increased lipid peroxidation and compromised ovarian antioxidant defence (Traber and Stevens, 2021). The significantly lower vitamin E levels observed in your PCOS participants support this mechanism and align with studies showing that insufficient vitamin E may contribute to poor follicular health, endocrine imbalance, and reduced ovarian responsiveness.

The lack of significant difference in vitamin A levels between groups reflects mixed findings in the literature. While some studies report lower retinol levels in PCOS women, others find no significant variation. The present results more closely align with the latter trend, indicating that vitamin A may be less sensitive to oxidative stress compared with vitamins C and E, or that its levels vary according to dietary and metabolic factors unique to each population.

The combination of elevated oxidative stress, reduced antioxidant vitamins, and disturbed reproductive hormones mirrors the mechanistic model in which oxidative and inflammatory pressures alter ovarian signalling pathways, impair granulosa cell function, and ultimately

contribute to hyperandrogenism and anovulatory cycles. The present findings therefore support and extend existing evidence that antioxidant imbalance plays a role in the hormonal abnormalities characteristic of PCOS.

4.4 Conclusion

The findings of this study demonstrate that women with polycystic ovary syndrome exhibit distinct biochemical abnormalities when compared with non-PCOS controls. These differences encompass elevated reproductive hormones, reduced antioxidant vitamin levels, heightened oxidative stress, and increased inflammatory activity. The PCOS group showed significantly higher concentrations of luteinizing hormone, testosterone, and a markedly elevated LH/FSH ratio, confirming profound gonadotropin dysregulation. Similarly, the substantially higher C-reactive protein levels indicate that systemic inflammation is an important component of the disorder.

Antioxidant profiling revealed that vitamins C and E were significantly lower in women with PCOS, suggesting compromised antioxidant defence. This depletion occurred alongside increased oxidative stress, reflected by elevated superoxide dismutase activity and reduced catalase function.

4.5 Recommendations

The biochemical patterns identified in this study suggest that several practical steps may be beneficial for improving the management and monitoring of women with polycystic ovary syndrome. Given the reduced levels of vitamins C and E observed in the PCOS group, it is advisable that clinicians consider routine evaluation of antioxidant status as part of the biochemical assessment of affected women. This may help identify individuals who would benefit from nutritional interventions aimed at strengthening antioxidant defences and reducing oxidative stress.

Because oxidative imbalance appeared alongside elevated reproductive hormones, dietary strategies rich in natural antioxidants may support endocrine regulation. Fruits, vegetables, whole grains, and vitamin-rich foods could be promoted as part of lifestyle counselling for women with PCOS. Where warranted, supervised supplementation of vitamins C and E may be considered, particularly in individuals who show marked depletion or present with symptoms suggestive of excessive oxidative stress. Such interventions should, however, be tailored and monitored to avoid excessive intake and ensure biochemical improvement.

The significant rise in inflammatory markers among PCOS participants indicates that inflammation is a relevant component of the disorder. Clinicians should therefore adopt a more integrated metabolic approach during evaluation, paying attention not only to hormonal irregularities but also to inflammatory and oxidative indices. Early identification of heightened CRP levels may help guide timely management strategies that address both metabolic and reproductive outcomes.

Given the strong hormonal disturbances observed, regular monitoring of LH, FSH, testosterone, and the LH/FSH ratio may provide valuable insight into the progression of endocrine imbalance in PCOS. Tracking these markers over time could also help assess the impact of therapeutic interventions, whether lifestyle-based or pharmacological.

Finally, the multifactorial nature of PCOS highlighted by this study underscores the importance of interdisciplinary care. Collaboration between biochemists, endocrinologists, nutritionists, and gynaecologists may provide more comprehensive management strategies that consider hormonal, metabolic, oxidative, and nutritional factors together.

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