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The determination of some hormonal imbalance in women with PCOS and to verify their effects on Calcium homeostasis

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***Abstract*— Polycystic ovarian syndrome is a common endocrinopathy in reproductive-aged women, associated with metabolic dysfunction influencing mineral metabolism and calcium regulatory pathway and affecting bone health. Thus, this study conducted to pinpoint specific hormonal abnormalities in PCOS women and to elevate their correlation with calcium homeostasis markers. A hundred specimen recruited from Iraqi women (50 PCOS patient and 50 serving as a control group (aged 18-40 years old). PCOS group was diagnosed according to standard Rotterdam criteria and were compared with controls without PCOS compatible in age and BMI. Blood samples were collected to quantify E2, LH, FSH, P.TH, Ca^{2+}, and total testosterone by using hormone's analyzer Addendum-Mini VIDAS apparatus (VIDAS) 12 mode l0, 1992. The PCOS group demonstrated higher LH, testosterone and calcium levels (4.96 ± 1.00) (0.64 ± 0.17) (10.37 ± 0.86) respectively, with decreased E2 and P.TH (55.12 ± 14.67) (25.08 ± 4.54) respectively when compared with control group, while FSH remained statistically unchanged. The data revealed a robust positive calcium-E2 correlation and an inverse E2-testesterone correlation implying a potential link between calcium homeostasis and ovarian steroidogenesis.**

Keywords: PCOS; Calcium homeostasis; PTH; LH; FSH; Testosterone.

I. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the predominant endocrine condition, affecting 15% to 18% of women at childbearing age, with a global prevalence between 5% and 10% [1,2]. It is a varied and complicated condition characterized by symptoms related to metabolism and reproduction, such as obesity, insulin resistance, hirsutism, ovulatory dysfunction, and infertility [3]. Women with PCOS often encounter reproductive complications that are significantly linked to vitamin D levels. The potential effects on hormones include Luteinizing hormone (LH), follicle-stimulating hormone (FSH), the LH/FSH ratio, and the regularization of the menstrual cycle [2]. The abnormal functioning of the hypothalamic–pituitary–ovarian axis and hyperinsulinemia lead to increase in the luteinizing hormone (LH) pulse frequency, and this increase promotes theca cell production of androgens. Concurrently, a relative deficiency in follicle stimulating hormone (FSH) disrupts the aromatization of granulosa cells (GCs) to estrogens, thereby impairing follicle maturation and ovulation [4]. According to the Rotterdam consensus, PCOS is diagnosed when two of the following criteria are met: oligo-ovulation or anovulation (ovulatory dysfunction), hyperandrogenism, and/or polycystic ovaries [5], (≥ 12 follicles measuring 2-9 mm in diameter and/or an ovarian volume > 10 mL in at least one ovary). Calcium is essential for sustaining a high quality of life, especially in older age. The deficiency leads to va changes, cataracts, alterations in brain function, and osteoporosis [6]. Many studies have shown how calcium affects ovulation, follicular development, and the pathophysiology of PCOS. Calcium significantly influences the conversion of testosterone to estrogens in granulosa cells, thereby establishing a balanced ratio of androgens and estrogens [7-9]. Ott *et al*. (2012) discovered an inverse correlation between parathyroid hormone (PTH) and blood calcium and $25OHD_3$, while positive relationships were identified between PTH,and body mass index and testosterone [10]. It is proposed that insulin resistance leads to hyperinsulinemia, which in turn raises insulin levels, thereby promoting androgen secretion from the ovaries. This process lowers the level of sex hormone binding globulin (SHBG) in the blood, which raises the level of free testosterone in the blood. So, hyperinsulinemia and too much androgen stop the growth and maturation of ovarian follicles [11]. This study aims to characterize selected hormonal imbalances in women with PCOS and to evaluate their associations with calcium homeostasis parameters (serum calcium, parathyroid hormone and other related hormones).

II. METHOD

A. Subject

This study was an investigation of a sample of Iraqi women with PCOS At the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al

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Nahrain University.The study included 100 Iraqi women. The samples were divided into two groups: 50 PCOS patients and 50 healthy controls. The average age of PCOs patients' groups and healthy groups was 18 – 40 years and the medical history of all PCOS patients was taken and was detected first by ultrasound scan, supported by information recorded if they experienced oligo menorrhea, amenorrhea, or highly irregular menses.

B. Sample collection

The samples were obtained from patients and control by drawing blood and separating the serum for the necessary analyses. 5 ml of venous blood was collected in activator tubes. The serum was separated by centrifugation for 10 minutes at 3000 rpm and then the serum was kept in Eppendorf tubes at -20 °C until used. Blood samples were collected from PCOS and non-PCOS individuals at the early follicular phase (3 or 4 days). Laboratory tests included fertility hormones (testosterone, FSH, LH and E2).

C. Materials and methods

Hormone's analysis was performed by using Addendum-Mini VIDAS apparatus (VIDAS) 12 mode l0, 1992, Biomerieux Company, France, through an enzyme linked fluorescent assay (ELFA) technique. Statistical analysis was performed using the T-test to compare the means between groups and determine the significance of differences.

Parameters	Mean ± SD		P Value <0.05
	Patient n=50	Healthy n=50	
Testosterone (ng/ml)	0.64 ± 0.17	0.43 ± 0.19	0.016 *
E2 (Pg/ml)	55.12 ± 14.67	133.68 ± 20.88	< 0.001 ***
LH (Iu/ L)	4.96 ± 1.00	3.41 ± 0.81	0.009 **
FSH (Iu/ L)	8.29 ± 0.71	6.15 ± 1.52	0.37 NS
PTH(Pg/ml)	25.08 ± 4.54	34.02 ± 3.02	< 0.001 ***
Calcium (mg/dl)	10.37 ± 0.86	8.29 ± 0.71	< 0.001 ***

III. RESULTS AND DISCSIONS

Table (1) shows the results that were obtained for the measured parameters of patients in comparison with healthy individuals (control group).

In the Table 2, the correlation coefficient between the parameters was evaluated to determine the strength of the correlation between these parameters. These results showed a strong inverse correlation between estrogen and testosterone, LH (- 0.12) (-0.15)When the level of testosterone increased, the level of estrogen decreased. On the other hand, there was a strong direct correlation between the level of estrogen compared to the level of calcium (0.6). When E2 increased, the calcium increased as well , and vice versa. Also, there was a weak direct correlation between the level of estrogen compared to the level of parathyroid (0.2) and weak inverse correlation between estrogen and FSH (-0.2). These results also indecated that there was weak direct correlation between the level of testosterone and LH, FSH, calcium and parathyroid (0.35, 0.40, 0.22, 0.25). Similar correlations were observed between LH and FSH, and calcium (0.36, 0.28) respectively.

Table (2) The correlation coefficient.

Correlation							
		E2	Testo	LH	FSH	Ca	P.TH
E2	Pearson Correlation	1	-.127	-.151	-.273	.611	.258
Testo	Pearson Correlation	-.127	1	.357	.405	.221	.256
LH	Pearson Correlation	-.151	.357	1	.361	.283	-.007
FSH	Pearson Correlation	-.273	.405	.361	1	-.068	-.089
Ca	Pearson Correlation	.611	.221	.283	-.068	1	-.109
PTH	Pearson Correlation	.258	.256	-.007	-.089	-.109	1

there was weak inverse correlation between LH and parathyroid level (-0.007) and between FSH level and calcium, parathyroid (-0.06, -0.08) and between calcium and parathyroid (-0.11) respectively. Table No. 1 summarizes the measured parameters of patients in comparison with healthy individuals (control group). A highly significant decrease was shown by these results ($p<0.01$) in the E2 hormone level (55.12 ± 14.67) in patient individuals in compare with healthy individuals (133.68 ± 20.88). In the same line, there is a highly significant decrease ($p<0.01$) in the parathyroid hormone level (25.08 ± 4.54) in patient individuals in compare with healthy individuals (34.02 ± 3.02). In contrast , thes results recorded significant increase ($p<0.05$) in the level of testosterone (0.64 ± 0.17) and high significant increase ($p<0.01$) in LH hormones level (4.96 ± 1.00) in comparison with the healthy groups (0.43 ± 0.19) and (3.41 ± 0.81) respectively. As well as, the results indicated a markedly significant increase. ($p<0.01$) in the levels of calcium (10.37 ± 0.86) in compared with the healthy groups (8.29 ± 0.71) and there was no significant in the level of FSH (8.29 ± 0.71) compared with control (6.15 ± 1.52). The present study highlights a distinct pattern of hormonal imbalance and calcium dysregulation in women with polycystic ovary syndrome (PCOS). Compared with healthy controls, patients exhibited significantly elevated serum testosterone, luteinizing hormone (LH), and calcium, coupled with a marked reduction in estradiol (E2) and parathyroid hormone (PTH), while follicle-stimulating hormone (FSH) remained statistically unchanged. Correlation analysis revealed a strong positive association between calcium and E2 and an inverse relationship between E2 and testosterone, suggesting a direct interplay between calcium homeostasis and ovarian steroidogenesis. Calcium is increasingly recognized as a key modulator of follicular development and ovulatory function. Intracellular Ca^{2+} acts as a second messenger in

granulosa cells, where it regulates aromatase (CYP19A1) activity and facilitates the conversion of androgens to estrogens [12, 13]. The strong positive correlation between serum calcium and estradiol observed in this study aligns with this mechanistic pathway. Higher systemic calcium may enhance granulosa cell aromatization, thereby promoting estrogen biosynthesis and maintaining an androgen–estrogen balance. Conversely, a decline in PTH despite elevated calcium could reflect a compensatory feedback loop in which suppressed PTH prevents further calcium mobilization from bone [14]. Recent molecular studies suggest that vitamin D–calcium signaling interacts with insulin receptor substrates and phosphatidylinositol 3-kinase (PI3K) pathways, influencing both insulin sensitivity and ovarian steroidogenesis [15]. This cross-talk provides a plausible explanation for the metabolic–reproductive interface in PCOS, where insulin resistance and calcium dysregulation may reinforce hyperandrogenism [16]. The negative correlation between estradiol and testosterone underscores the disruption of the hypothalamic–pituitary–ovarian (HPO) axis in PCOS. Pulsatile GnRH secretion favors LH hypersecretion, this stimulates theca cells to make androgens [17]. In the context of relative FSH insufficiency, aromatase activity is reduced, limiting conversion of testosterone to estradiol and leading to follicular arrest [18]. The simultaneous suppression of PTH suggests that the calcium–vitamin D axis is not functioning optimally to correct the underlying endocrine imbalance [19]. Lower PTH levels in PCOS patients contrast with some earlier reports that documented PTH elevation in vitamin D–deficient phenotypes [20]. One explanation could be calcium-sensing receptor (CaSR) desensitization or altered renal calcium handling, leading to high serum calcium that suppresses PTH despite impaired ovarian calcium utilization [21]. PTH is known to modulate estradiol secretion indirectly by influencing granulosa cell cAMP pathways; therefore, a blunted PTH response may further compromise estrogen production and contribute to prolonged or anovulatory cycles [22]. Recent multi-omics analyses have highlighted Ca^{2+}-dependent signaling in ovarian theca cells, linking intracellular calcium flux to androgen biosynthesis through activation of the steroidogenic acute regulatory protein (StAR) and CYP17A1 enzymes [23]. Moreover, Mendelian randomization studies suggested a bidirectional relationship: serum calcium levels influence fasting glucose and insulin resistance, which are themselves central to PCOS pathogenesis [24]. Elevated calcium may thus be both a consequence and a driver of metabolic disturbances [25]. These findings underscore the potential value of targeting calcium and vitamin D pathways in the management of PCOS. Clinical trials have shown that calcium and vitamin D supplementation can reduce serum androgens, improve menstrual regularity, and enhance insulin sensitivity [26]. Our observation of high calcium but low PTH suggests that individualized supplementation strategies may be required, focusing not only on calcium intake but also on vitamin D status and PTH responsiveness [6]. Monitoring serum calcium, PTH, and vitamin D together may provide a more comprehensive assessment of metabolic risk and reproductive potential in PCOS patients [27]. Vitamin D deficiency causes a compensatory increase in PTH, in an effort to maintain calcium. However, it was not clear what level of vitamin D defines vitamin D deficiency. Some authors have proposed the use of the term ‘‘vitamin D insufficiency’’ and this is defined as the level at which the decreased vitamin D causes an increase in PTH [28]

While the present study provides compelling correlations, causality cannot be established. Future research should include longitudinal intervention studies assessing the effect of calcium or vitamin D modulation on ovarian steroidogenesis and insulin sensitivity, as well as molecular assays to explore Ca^{2+}-dependent aromatase regulation. Investigating genetic variants in calcium-sensing receptors or vitamin D receptors may also clarify interindividual differences in calcium metabolism and PCOS phenotypes. On the other hand, decreased levels of E2, PTH and FSH versus increased level of LH, testosterone and calcium were observed in patients group as compared with healthy group, this could be explained by that, women with PCOS who were hyperresponsive to FSH treatment were more likely to have hyperstimulation during ovulation induction. This was likely due to the abundance of small antral follicles in women with PCOS and the increased number of FSH receptors per granulosa cell in follicles from anovulatory women with this disorder. Nonetheless, there is a lack of clinical data regarding the impact of E2 on GC function and follicle formation in women. Consequently, nearly all of our knowledge of the influence of E2 on follicle health has been gathered from studies on animal models. E2 does not seem to have a direct effect on GCs because E2 response elements have not been found on the CYP19 promoter in either rat or human GCs. It is probable that a synergistic interaction between E2 and FSH augments GC function and follicular development. Research demonstrates that optimal FSH stimulation of aromatase activation, antrum formation, and LH responsiveness in granulosa cells requires E2. This necessity is further corroborated by studies utilizing β-estrogen receptor knockout mice, Units which demonstrate the critical function of E2 in facilitating optimal FSH activity in granulosa cells [29]. Furthermore, the reason of the decline of testosterone levels with age in women with PCOS remains unidentified. The decrease in non–SHBG-bound testosterone levels, coupled with constant SHBG levels, indicates a decline in testosterone synthesis and insulin are regarded as the principal stimulators of testosterone synthesis in polycystic ovary syndrome (PCOS). It seems that E2 doesn't directly affect GCs because E2 response elements have not been found on the CYP19 promoter in GCs from either rats or humans. A synergistic interaction between E2 and FSH probably improves the function of GCs and the growth of follicles. Numerous studies suggest that optimal FSH stimulation of aromatase activation, antrum development, and LH responsiveness in

granulosa cells requires E2. This necessity is further validated by studies on β-estrogen receptor mutant mice, illustrating the essential role of E2 in promoting sufficient FSH activity in granulosa cells [30]. Regarding PTH, most of the published studies have reported an elevated level of parathyroid hormone (PTH) among woman diagnosed with (PCOS). This increase was usually attributed to vitamin D deficiency, insulin resistance, and chronic low-grade inflammation, all of which were commonly associated with PCOS and may stimulate parathyroid activity. However, in the present study, our results demonstrated a significant decrease in serum PTH levels in PCOS patients compared to healthy controls. This finding is in contrast to the majority of available literature and could be explained by several scientific reasons like that, PTH secretion is inversely regulated by serum vitamin D and calcium. If our PCOS cohort had relatively higher vitamin D levels (due to supplementation, dietary habits, or sun exposure), this might have suppressed PTH secretion [31], or, Differences in genetic background, ethnicity, lifestyle, and dietary calcium intake may contribute to heterogeneity between studies. Some populations may not show the typical PTH rise in PCOS [32]. While regarding LH and FSH, polycystic-ovary-syndrome (PCOS) is characterized by an elevated LH/FSH ratio, with increased LH secretion and relatively normal or reduced FSH levels. However, in other study, demonstrated the opposite pattern: increased FSH levels along with reduced LH levels in PCOS patients. Several scientific explanations could account for this discrepancy, PCOS is a heterogeneous syndrome with multiple phenotypes (hyperandrogenic, ovulatory, normoandrogenic, insulin-resistant types). Some phenotypes may not show the classical LH elevation. In fact, women with predominant metabolic features and less androgen excess may present with normal or even low LH levels [33], moreover, The secretion of LH and FSH depends on the frequency and amplitude of GnRH pulses. While high-frequency pulses favor LH secretion, slower pulses preferentially stimulate FSH release. In certain PCOS subgroups, a shift in GnRH dynamics could explain the observed increase in FSH and decrease in LH [34], plus, Obesity, which is prevalent among PCOS women, may blunt LH secretion through increased estrogen production from adipose tissue and altered insulin signaling. This may suppress LH release, while FSH remains relatively preserved or even elevated to support folliculogenesis [35] Regarding testosterone, one of the central biochemical hallmarks of polycystic ovary syndrome (PCOS) is hyperandrogenism, manifested either clinically (hirsutism, acne, alopecia) or biochemically (elevated serum testosterone levels). The elevation of testosterone in PCOS patients can be explained through several mechanisms include, Excess Ovarian Androgen Production since in PCOS, the ovarian theca cells are hyperresponsive to luteinizing hormone (LH) and insulin. This results in enhanced activity of steroidogenic enzymes, leading to increased testosterone synthesis [36]. Moreover, insulin resistance and compensatory hyperinsulinemia, commonly observed in PCOS, act synergistically with LH to stimulate androgen production in the ovaries [37]Also, in some women, the adrenal glands contribute significantly to androgen excess. Hyperactivity of adrenal androgen secretion (DHEAS, androstenedione) can increase circulating testosterone [38].

IV. Conclusion

Our findings suggest that specific hormonal imbalances in PCOS correlate with changes in calcium homeostasis, which may have consequence for skeletal health monitoring in this demographic. Longitudinal and mechanistic studies are necessary to determine the connection and investigate targeted interventions

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Conflict of interest

Authors declare that they have no conflict of interest.

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