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# Studying the relationship between mTOR activity and fertility hormones imbalances in polycystic ovary syndrome in Basra city

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***Abstract*—Polycysticovariansyndrome (PCOS) is an endocrine-related condition that is world's leading cause of infertility affects women of reproductive age. The current study set out to investigate the effect of mTOR functions on PCOS women and its relation to fertility hormones. Total of 62 blood samples were collected, 42 of them were gathered from those who had recently been diagnosed with PCOS and 20 samples were collected from healthy women. The levels of mammalian target of rapamycin (mTOR) were measured using the (ELISA) technique while prolactin and testosterone were measured by Cobas e411. The study found a significant decrease in mTOR levels and a significant increase in prolactin and testosterone levels in women with PCOS compared to healthy women. Regarding age and BMI groups, no discernible variations were detected. This study concluded that dysregulation of mTOR is linked with PCOS, in addition, mTOR has a negative relationship with prolactin.**

***Keywords*—PCOS, mTOR, prolactin, testosterone, immunoregulation.**

## I. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a common endocrine and metabolic condition affecting around 6-20% of women who are of reproductive age. Early in puberty, the majority of PCOS symptoms manifest [1]. PCOS has several etiological factors, but little is known about the molecular mechanisms and signaling pathways behind the complex pathophysiology, which includes several pathways and proteins [2]. A key factor in the pathophysiology of PCOS is ovarian dysfunction, which includes aberrant steroid hormone production and follicular halt. Hyperandrogenism is one of the key characteristics of PCOS [3].

Anovulation in women is most commonly caused by two conditions: hyperprolactinemia and PCOS [4]. Prolactin is a pituitary hormone that is mostly recognized for its function in promoting the growth and development of mammary cells, which is essential for maintaining the restricted and irregular metabolic balance during breastfeeding [5]. Previous studies have mentioned that there was a pathophysiological connection between PCOS and hyperprolactinemia since the 1950s [4] Furthermore, they linked that to several processes, including immunological and reproductive system control [6]. However, in the recent studies they sound that prolactin function is linked to metabolic balance through its interactions with the pancreas, liver, hypothalamus, and adipose tissue. Women who have PCOS may have changed prolactin levels [7]. Since PCOS and hyperprolactinemia are common endocrine disorders, it is impossible to rule out a coincidental relationship [4]. The most popular theory to explain the connection between hyperprolactemia and PCOS is according to the theory that PCOS generates relative hyperestrogenemia, which in turn causes hyperprolactinemia [8]. In fact, number of experimental investigations have demonstrated that prolactin production rises in response to estrogen [9].

The two primary androgens in the PCOS patients are testosterone and androstenedione, which also served as a diagnostic marker for excess ovarian androgens [10]. PCOS causes a noticeable rise in androgen, which contributes to problems with fertility. Hyperinsulinemia and insulin resistance are strongly linked to androgen overexposure [11]. PCOS is characterized by persistent low-grade inflammation, immune and metabolic disorders are present in the inflammatory environment, several other organ systems are also lately being affected in addition to the female reproductive system, by the pathogenic role of immunological dysregulation in PCOS, Insulin resistance and hyperandrogenism are associated with immune cell dysfunction [12]. MTOR, The protein kinase, regulates a variety of biological processes including autophagy, immunological responses, cellular metabolism, proliferation, and migration in order to preserve cellular homeostasis [13]. MTOR appears to be significantly more involved in female reproduction than just a basic positive or negative trigger. Under physiological settings, variety of variables work together to regulate mTOR in order to control the course of many processes, including ovulation, endometrial alterations, folliculogenesis, and embryonic development [14].


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Recent publications deal with the mechanisms behind the role of mTOR in female reproduction under physiological and pathological conditions [4]. In order to better understand the mTORr's role in PCOS women, the current work focused on investigating the relationship between some fertility hormones (prolactin and testosterone ) and mTOR

## II. MATERIALS AND METHODS

### A. *Design of study*

The study was conducted as a case-control observational study. After outlining the study objectives and evaluating patient satisfaction, two study groups were formed. The first group consisted of forty-two newly diagnosed women with PCOS who attended private maternity clinics in Basra City between December 2022 and July 2023. These women are either single or married. Without specification, they just met the inclusion criteria, which included being female patients with PCOS for at least three months, aged between 18 and 45, and not having any other immune system disorders, diabetes, or pregnancy. While the second group consisted of twenty healthy women classified as the control group. The age groups were divided into four groups, in the age range of 6 years old within each group, the groups are as follows: less than 20, 20–26, 26–32, and 32 and more. While BMI patients group was divided into two groups: the first included patients with a BMI less than or equal to 25, and the second consisted of patients with a BMI more than 25.

### B. *Samples Collection and Biochemical Assays*

A volume of 3ml of venous blood samples were collected, consequently, sera were separated and kept at -20 ºC until use. The level of mTOR was assessed using the ELISA technique according to the manufacturer's guidelines (Cloud-Clone-Corp, USA). Testosterone and prolactin were tested by (Cobas e 411, Basel, Switzerland). The study proposal was approved by the Ethics Committee of the College of Pharmacy, University of Basra, Iraq, with the approval code (EC56).

### E. *Statistical Analysis*

The statistical analysis of the data was conducted using SPSS version 26. For parametric data, an independent t-test was employed; for non-parametric data, a Wilcoxon test and a Mann-Whitney U test were used for comparison. The mean ± standard deviation (SD) was used to show the results. $P < 0.05$ was used to indicate a statistically significant difference between the groups.

## III. RESULTS

### A. The comparison levels of mTOR, prolactin, and testosterone between PCOS women and the control group.

Table 1 shows the level of prolactin, testosterone reveals significant elevation (P-value 0.000) in newly diagnosed PCOS women compared with healthy individuals. Conversely, there was a significant dropping in mTOR serum level than the control.

Table 1: The levels of mTOR, prolactin, and testosterone in PCOS women and a control group

| Study markers | *Groups* | *No.* | *Means* | *SD* | *P-value* |
|---|---|---|---|---|---|
| mTOR( ng/ml) | Patients | 42 | 0.40 | 0.31 | 0.000* |
| | control | 20 | 2.51 | 0.78 | |
| Prolactin (ng/ml) | Patients | 42 | 19.84 | 10.76 | 0.005* |
| | control | 20 | 13.91 | 7.59 | |
| Testosterone (ng/ml) | Patients | 42 | 1.14 | 0.72 | 0.000* |
| | control | 20 | 0.85 | 0.19 | |

*significant differences

### B. The comparison levels of mTOR, prolactin, and testosterone within PCOS regarding age groups

Table 2 shows there were no significant differences ($P>0.05$) in the levels of mTOR, prolactin, and testosterone among the different age groups.

Table 2: The levels of mTOR, prolactin, and testosterone within PCOS

| Study markers | *Age groups* | *No* | *Means* | *SD* | *P-value* |
|---|---|---|---|---|---|
| mTOR (ng/ml) | less than 20 | 6 | 0.61 | 0.33 | 0.21 |
| | 20-26 | 13 | 0.30 | 0.24 | |
| | 26-32 | 13 | 0.44 | 0.36 | |
| | 32-more | 10 | 0.36 | 0.29 | |
| Prolactin (ng/ml) | less than 20 | 6 | 16.25 | 11.09 | 0.08 |
| | 20-26 | 13 | 20.12 | 10.85 | |
| | 26-32 | 13 | 20.98 | 10.01 | |
| | 32-more | 10 | 20.34 | 12.76 | |
| Testosterone (ng/ml) | less than 20 | 6 | 1.50 | 0.94 | 0.18 |
| | 20-26 | 13 | 1.20 | 0.85 | |
| | 26-32 | 13 | 1.06 | 0.62 | |
| | 32-more | 10 | 0.95 | 0.48 | |

women regarding age groups

*significant differences

### c. The comparison levels of mTOR, prolactin, and testosterone within PCOS women regarding BMI.

As noted in table 3, mTOR, prolactin, and testosterone levels were not significantly altered according to BMI groups.

Table 3; The levels of mTOR, prolactin, and testosterone levels within PCOS women regarding BMI.

| Study markers | *Groups* | *No* | *Means* | *SD* | *P-value* |
|---|---|---|---|---|---|
| mTOR (ng/ml) | ≤25 | 23 | 0.40 | 0.30 | 0.32 |
| | > 25 | 19 | 0.49 | 0.34 | |
| Prolactin (ng/ml) | ≤25 | 23 | 19.72 | 10.14 | 0.32 |
| | > 25 | 19 | 16.86 | 10.46 | |
| Testosterone (ng/ml) | ≤25 | 23 | 1.19 | 0.83 | 0.15 |
| | > 25 | 19 | 1.31 | 0.62 | |

*significant differences

c. The relationship of mTOR with prolactin and testosterone

Prolactin was significantly correlated with mTOR in a negative weak correlation (r=-0.338, P=0.033), However, testosterone was not correlated with mTOR: table 4 and Figure 1(A,B).

Table 4; The correlation of mTOR with prolactin and testosterone

| Study markers | Prolactin | | Testosterone | |
|---|---|---|---|---|
| | *r* | *P-value* | *r* | *P-value* |
| mTOR | -0.338 | 0.033* | 0.289 | 0.071 |

*significant differences

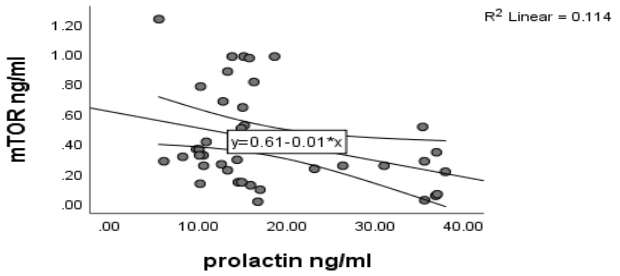


Figure 1A

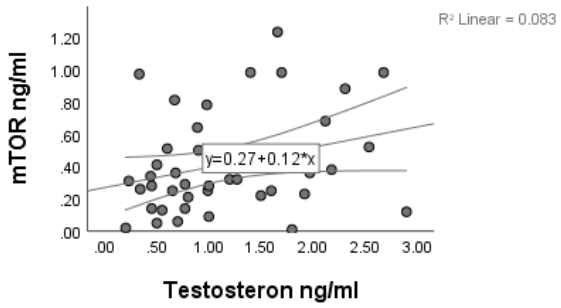


Figure 1B

Fig. 1: The correlation of mTOR with prolactin(A) and testosterone (B).

## IV. DISCUSSION

The present study focused on investigation the mTOR status in women afflicted with PCOS. Also, it aims to explore the prolactin and testosterone profile and how these effects may vary across different age groups and BMI categories, in addition to investigating the mTOR relationship with the mentioned hormones. The results of this study showed a significant decrease in mTOR levels compared to the control group. Regarding age and BMI groups, mTOR showed no significant alteration within these groups.

According to Liu et al. [15] study showed significance of mTOR in cell growth, proliferation, and differentiation. PCOS is one of the many diseases that are thought to be related to the over-activation of the mTOR pathway [15]. mTOR signaling has two remarkable purposes in the ovary. First, there is increased mTOR expression in oocytes, which has a lot of promise for ovarian reserve protection applications. Second, oocyte meiotic maturation is hampered by mTOR suppression, which may restrict the application of mTOR-suppressing medications for disorders of fertility, Moreover, mTOR seems to be essential for endometrial alterations and hypothalamic functioning in the ovary [14]. According to a study conducted by Wang et al. [16] that referred to defective PI3k/AKT/mTOR signaling is linked to low insulin levels and insulin resistance in PCOS ovaries and the endometrium. This pathway is typically necessary for cell proliferation and the control of inflammatory processes in all cells [16]. Zhang et al. [17] discussed how the endometrium of patients with PCOS had an overactive phosphoinositide-3-kinase / Protein kinase B (PI3K/AKT ) signaling pathway. Moreover, insulin resistance was most likely a major contributing cause to the overactivation of PI3K/AKT in PCOS endometrium [17]. Those studies come in the same line with the present study. AKT may either directly or indirectly activate the negative regulator complex namely tuberous sclerosis proteins TSC1/TSC2, which in turn stimulates the whole signaling cascade [18]. Because that can work in the upstream molecule of the PI3K/AKT/mTOR pathway to limit the activation of mTOR [19]. In another study, PCOS patients' luteal GCs had the same quantities of mTOR protein as healthy women. However, upon stimulation with insulin, luteal GCs with PCOS have lower levels of mTOR protein expression than do healthy individuals [20]. On the other hand, rapamycin's suppression of the mTOR signaling pathway would block the gonadotropic axis throughout puberty, lower LH and estradiol levels, significantly delay vaginal opening, and result in ovarian and uterine atrophy [21]. While Li et al. [22] revealed that a high-fat diet prevents autophagy by blocking AMPK phosphorylation and encouraging mTOR to transition to phosphorylation [22].

Studies has shown that mTOR signaling regulates female reproduction through several ovarian functions, including ovarian reserve and aging, oocyte meiotic maturation, follicle development, and the proliferation of ovarian somatic cells [14]. To control metabolism and energy balance, the mTOR is sensed by hormones, nutrition, and energy signals. The hypothalamus's mTOR activity, which is linked to fluctuations in energy status is essential for controlling food intake and body weight [23]. The pool of developing antral follicles shrinks as women age, menopause is caused by ovarian follicle counts dropping below a certain threshold, and it is an irreversible process [24]. Tufan et al. [25] suggested that according to hormonal and ultra-sonographic testing, the pattern of reproductive aging does not seem to indicate a sudden change at a certain age, but rather shows a steadily growing rate of decrease in the third decade of life [25].

In our current study, the focus was on the effect of PCOS on two important hormones related to female fertility: prolactin and testosterone. The study found a significant increase in the serum levels of both hormones when comparing women with PCOS-healthy women. However, there were no significant differences in serum levels of these hormones when comparing based on age and BMI.

A study done by Shenta et al. [26] found that the hormone profile was extremely varied in prolactin levels, raised levels of hormones greater than usual, lipid profile, and antioxidant variables can disrupt the ovarian system and

lead to PCOS [26]. Another study supports present finding indicated that women with PCOS have considerably greater prolactin levels than women without PCOS [27], these two studies agree with our present results .

On the other hand, Barrea et al. [28] improved that PCOS women had elevated levels of testosterone [28]. As well as Sharquie et at. [29] demonstrated that free testosterone is considered a positive marker for the diagnosis of PCOS [29]. While the majority of PCOS patients have excess androgen production from the ovaries. Over 50% also have increased androgen production from the adrenals, which is shown as elevated serum dehydroepiandrosterone levels [30]. Adrenal androgens have little to no androgenic activity, but they do contribute to the pathophysiology of hyperandrogenism by being converted to testosterone in the peripheral circulation [31]. From previous studies, it found that women with PCOS experienced a decrease in serum androgen levels as they advance age [32,33]. Extenuation of hyperandrogen most likely results from a concurrent reduction in the pool of developing antral follicles, reduction in adrenal gland production, and pancreatic cell exhaustion [34, 35]. Along follow-up study on the effect of age on hormones on PCOS done by Jacewicz-Święcka et al. A study done by [36] agrees with the present study, and it revealed that the prevalence of biochemical and clinical hyperandrogen did not change significantly with aging, but the prevalence of PCO-morphology decreased [36]. According to Ernst et al. [37] who demonstrated neither any systematic changes in baseline hormone concentrations following major weight loss, nor any meaningful correlation between basal prolactin levels and the degree of obesities or associated metabolic disorders [37]. On the same hand, Gray et al. [38] found that following weight loss, prolactin levels in overweight and obese premenopausal women did not decrease [38]. Previous studies showed that shorter trials in weight reduction had similar changes in testosterone level lowering as longer trials. Therefore, the reductions in testosterone did not appear to be connected to the length of the intervention and the amount of weight loss did not seem to be correlated with the degree of the testosterone drop [39, 40, 41]. Other studies approved that there were lowering of testosterone levels in obese females with PCOS after weight loss [42, 43].

## V. CONCLUSION

This study concluded that the dysregulation of immunoregulatory marker mTOR is linked to the PCOS, and causes elevation in fertility hormones. Furthermore, this study gives evidence that these markers are not age neither BMI related. Finally, the study found that there is no correlation between mTOR and testosterone, but it negatively correlated with prolactin.


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## ETHICAL CONSIDERATION

Permission had been obtained from every participant to conduct ethical research, and gynecologists at private clinics in Basra city assisted with the patient selection process.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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